STUDIES ON MODIFICATION OF PHOSPHORUS CONCENTRATION OF DIETS, HYDROLYSIS OF PHYTATE BOUND PHOSPHORUS, AND EXCRETION OF PHOSPHORUS BY DAIRY COWS

By

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This dissertation is dedicated to my dearest parents:

you gave me life,
you taught me discipline,
you encouraged me, you guided me,
and most of all you never stopped loving me
I could ask for nothing more.

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Abstract of Dissertation Presented to the Graduate School
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AND EXCRETION OF PHOSPHORUS BY DAIRY COWS

BY

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Results of a trial conducted on five commercial dairies showed that as concentration of phosphorus (CON P) in concentrate portion of diet decreased estimated total excretion of P decreased. It was not known if reduced intake of P affected voluntary intake of feed (VIF) or milk yield (MY) of dairy cows.

Twelve cows were fed 20 kg of dry matter (DM) containing .41% P daily for four wk (wk -4 through wk -1). Cows then were assigned randomly to one of three diets: 30 (L), .41 (M), or .55% (H) P in diet DM from wk 1 through wk 9. Collection of excreta (milk, feces, urine) occurred at wk -1, 3, 6, and 9. At wk -1 cows excreted 88.2% of P consumed daily. Phosphorus was excreted in feces (68.6%), milk (30.2%), and urine (1.0%). When results from wk -1 to wk 9, were compared, cows assigned to L had decreased intake (27%) and excretion of P in feces

(from 53.7 g/d to 41.6 g/d, 22.5%). Cows assigned to H increased intake 48.8% and excretion of P in feces 48.6%.

Twenty-four cows were used in an incomplete randomized block design with three periods and six diets: three CON-P (.33, .43, and .54%) and two concentrations of Ca (.60 and .97%). Calcium:phosphorus ratios between 1.1:1 and 2.9:1 and CON-P in diet did not affect V1F or MY, but there was a nonsignificant trend for decreased MY (22.8, 22.6, and 21.9 kg of milk/d) as CON-P in diet increased from .33 to .54%. As concentration of Ca decreased from .97 to .60% yield of 3.5% fat corrected milk increased from 24.1 to 25.0 kg of milk.

Greater than 95% of phytate bound P (Phy-P) in most concentrate feeds disappeared from feed solids (was hydrolyzed or solubilized) when feeds were incubated individually in vitro for 8 hr. Greater than 99% of Phy-P in solution was hydrolyzed from most feeds between six to 12 hr. Hydrolysis of Phy-P in cottonseed meal and in solution occurred between 12 and 24 hr. Less than 1% of Phy-P in feed was recovered in feces.

Dietary CON-P at .33% provided adequate P to maintain V1F and MY during short term studies. Phytate bound P in feedstuffs appeared to be available for use by lactating cows.

CHAPTER 1
INTRODUCTION

In Florida in order to maintain high quality fresh water it is necessary to minimize contamination of surface and ground water sources. Typically, major contaminants arise from residential, industrial, or agricultural sources. In North Florida, attention has centered around contamination of ground water, whereas, in South Florida, attention has centered around contamination of surface water. Increased population, decreased level of water table, and increased infiltration of salt water makes the supply of fresh water a critical issue.

The Floridan aquifer provides water for much of Florida. This aquifer is located under several hundred meters of sedimentary strata and reaches from the Panhandle region (northwest) as far south as the everglades (west coast) and Vero Beach (east coast). The Biscayne aquifer underlies an area of about 7,500 square kilometers in Dade, Broward, and south Palm Beach Counties (southeast coast). In this aquifer, lateral salt water intrusion is prominent due to usage which has exceeded recharging of the water table during the last 50 yr. Intrusion of salt water can be reduced by recharging the Biscayne aquifer from local rainfall and from canals draining water conservation areas (Water Resources Council, 1977). A key water source to recharge the Biscayne aquifer is Lake Okeechobee. The general health and well

being of Lake Okeechobee has a direct effect on maintenance of a fresh water supply for Florida s southeast coast.

If agriculture is a part of the problem then agriculture must be a part of the solution. The problem of concern is concentration of phosphorus (P) in Lake Okeechobee. This 1,900 square kilometers lake located in South Florida ranges from 5 to 6 meters deep, supplies drinking water to communities around the lake, serves as reserve water for Florida's southeast coast, and provides water to the Florida everglades. The lake also serves as a recreational facility and provides some of the best sports fishing in the United States.

A large population of dairy cattle (> 35,000 lactating cows) resides north of Lake Okeechobee in the Taylor Creek-Nubbin Slough watershed (Figure 1-1). Nutrient rich runoff from farm lands enters creeks and streams (Graetz, 1989). The water eventually flows into the lake. Included in these nutrients is P, the nutrient identified as a major contributor to algal growth which in turn is a causal agent for subsequent eutrophication of the lake. As concentration of P in the lake water increases, algal growth increases. Increased turnover of algae occurs with increased decomposition of algae. Although algae are photosynthetic during daylight hours, they require oxygen during darkness. This demand for oxygen, combined with the demand for oxygen associated with decomposition of dead organic matter, depletes the water of oxygen. Although large growths in algae populations (blooms) are common in lakes, it was in 1986 that Mycrocystis was identified. This is an algae which appears in lakes undergoing eutrephication.

High concentrations of P in nutrient rich water entering from Taylor Creek-Nubbin Slough watershed have been traced to the dairies as

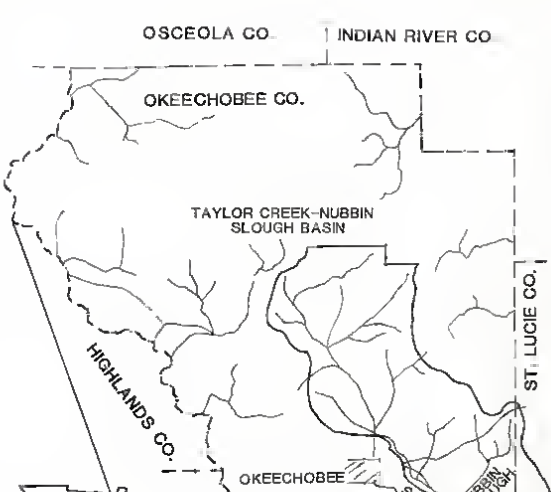


Figure 1-1 Okeechobee and neighboring counties. Most of the 108,000 acre Taylor Creek-Nubbin Slough Basin is located in Okeechobee County with small portions of the basin extending into Martin and St. Lucie Counties. Figure obtained from Woods (1986).

the source of origin (Graetz, 1989). Dairy operations in Okeechobee County and surrounding areas have utilized advanced technology and innovative management techniques. Currently, the survivability of the dairy industry is related not only to financial management but also, in part, to the ability of dairymen to further implement innovative techniques to reduce the runoff of P from land used for dairy purposes (Harvey, 1989).

Phosphorus utilization by the animal is partitioned to lactation, gestation, and body function, and excretion primarily occurs through feces, milk, and urine. Animal requirement for P varies according to body size, stage of lactation, stage of gestation, amount of intake and availability of P in feedstuffs. Correct feeding of dairy cattle is essential to maintain milk production, reproduction, and good physical condition.

Diets of dairy cattle in Okeechobee county have been formulated to provide essential nutrients at minimal cost. As an essential nutrient, attention has focused on concentration of P in diets. By-product feeds, primarily those left over from processing of human food, are key components. For instance, a by-product of orange processing is citrus pulp and of cottonseed oil extraction is cottonseed hulls. Each by-product feed has its own nutritive value. Use of individual ingredients is dependent upon availability, cost, nutritive value, handling ability, and willingness of cow to consume it (acceptability).

During the early 1960s, large quantities of citrus pulp (up to 50% of diet dry matter (DM)) were included in diets. This resulted in excess intake of Calcium (Ca) with respect to intake of P. This resulted in increased occurrence of milk fever. The Ca:P ratio greatly

exceeded the recommended ratio of 2:1. To combat high incidence of milk fever, concentration of dietary P was increased which simultaneously reduced the Ca:P ratio.

Inorganic mineral supplementation generally was used to increase concentration of P in diets. Historically, P has been the most expensive mineral to supplement in diets for dairy cows. In addition to supplementation of P, dairymen imposed a constraint of no more than 30% of diet DM as citrus pulp. Citrus pulp has a high concentration of Ca (2.07% of DM) and a low concentration of P (.13% of DM). This constraint helped prevent feeding of high concentrations of dietary Ca and reduced the need for inorganic phosphorus supplementation of P.

By the 1970s, P again became a concern to those individuals formulating diets for dairy cattle. Research in simple stomached animals indicated P bound to phytate (myo-inositol hexaphosphate; Figure 2-1) was unavailable to the animal. Consequently, concentration of P in diets fed to dairy cattle was increased.

Prior to 1986, the average formulated concentration of P in diets for lactating dairy cattle in South Florida ranged from .45 to .60% of diet dry matter (DM). The National Research Council committee for establishing nutrient requirements of dairy cattle (National Research Council, 1978) recommended a concentration of .31 to .40% of diet DM as P for lactating dairy cows, depending on animal size and status of production. Thus, the concentration of P in diets of lactating dairy cattle in South Florida were being formulated at 12 to 50% greater than recommended concentrations.

This dissertation will address intake of P in dairy cattle relative to their needs. The objectives were

1) to evaluate effects of concentration of P in concentrate portion of diets on concentration of P in feces of lactating deiry cows;

2) to quantify pathway of excretion (milk, feces, and urine) and determine effects of concentration of P in diet on excretion of P by lactating deiry cows when feed intake was restricted,

3) to determine effects of concentration of dietary P and Ca on voluntery feed intake and milk production; and

4) to quantify disappearence of P bound to phytate *in vitro* from feedstuff soiids and subsequent disappeerence from artificial rumen environment

CHAPTER 2
REVIEW OF LITERATURE

A series of metabolic sequences enable animals to consume, digest, absorb, and excrete dietary elements. Each of these can be considered a complex task when approached from the cellular level. Numerous essential nutrients must be absorbed by the animal to ensure survival. The nutrient of interest in this review is P.

Phosphorus does not occur naturally in its free state. It combines spontaneously with other elements. The most abundant form of P is as inorganic phosphate (PO_4) noted as P_i. In mammals, 80% of P in the body is found in bone as calcium hydroxyapatite ($3Ca_3(PO_4)_2 \cdot Ca(OH)_2$). In the form of adenosine mono-, di-, and tri-phosphates, P forms high energy bonds. These bonds store and release energy needed for metabolism. In addition to its contribution as an energy source, P participates in protein formation, is a component of phospholipids (essential in membrane development), is important in nucleic acid synthesis, as well as being a key component in many coenzymes (Irving, 1964).

Forbes (1937) summarized the complexity of the role of P in metabolism:

> In views of the conception of complete nutrition, and the obvious facts that no essential nutrient can be more essential than another, but that there are inevitable differences in the relative extent of the body reserves of essential nutrients, and in the rapidity with which they may be physiologically mobilized, the noticeable effects of differences in the level of P supply may not be definitely specific, in the sense of being obviously related either to its chemical nature or to the more conspicuous of its functions, but may be general in

character. In fact, in a critical sense, there is no such thing as P metabolism, there is only metabolism in general, in which P is involved--in a vast diversity and complication of ways. (p. 421).

Availability, Absorption, Retention, and Excretion of Phosphorus in the Digestive Tract

Availability

Presence of nutrients in the digestive tract is necessary, yet not always sufficient for absorption. Before a required nutrient can be of nutritional value, it must be in a form that can be digested, absorbed, and transported to the part of the body where it is utilized for its essential function. The term biological availability is used as a measure of the ability of the element or ion to support some physiological process.

Chemical form, natural chelators, and physiologic status can render nutrients unavailable for absorption. The total content of an element in a particular compound or in a complete ration has little significance unless it is qualified by a factor indicating the biological availability of the element to animals. Chemical analysis shows how much of a given mineral nutrient is present, but it does not indicate to what degree, if any, the nutrient is utilized when it is consumed by animals. Supplementation of P has yielded varied benefits depending on source of supplementation and its availability.

In a review of biological availability of minerals in feeds Peeler (1972) identified factors that influence the utilization of P for various species of animals. These included type of ration fed, chemical form of element, Ca:P ratio, age of animal, sex, concentration of fat and energy in diet, plane of nutrition, environment, hormones, disease

and parasites, concentration of protein and trace elements, interactions with other minerals and nutrients, chelating agents, the physical nature of sources of P and other feedstuffs in the diet (especially particle size), feed processing and numerous others. Many of these factors become important when nutrients in diets are low or deficient. Chemical form and availability of P has received the greatest consideration.

Phosphorus is ingested in two forms. These are P which occurs naturally in dietary ingredients (organic) and P which is added to feedstuffs as a supplement (inorganic).

Organic. Organic sources of P include phospholipids, proteins, nucleic acids, and phytate. Lofgreen and Kleiber (1953, 1954) determined the availability of P from chopped alfalfa hay for wether lambs. Excretion of metabolic P was determined by injection of radiolabelled P (^{32}P) into the blood stream. Fecal P, which originated from the body, was separated from that which passed through the gastrointestinal tract (GIT). The latter fraction represented P unavailable for digestion and absorption. Eighty-eight percent of P excreted in feces was of metabolic origin. True digestibility of P was 91% (Lofgreen and Kleiber, 1953). In a second experiment using mature wethers, Lofgreen and Kleiber (1954) reported that 91% of P excreted in feces was from endogenous sources, and true digestibility of P was 94%.

Estimates of true digestibility in wether lambs consuming alfalfa hay was greater than estimates of true digestibility of P in a ration consisting of equal parts alfalfa hay and mixed concentrate, when fed to two lactating dairy cows (91 and 94% vs 50 and 64%) (Kleiber et al., 1951). This possibly indicated a reduced availability of P from concentrate sources for lactating dairy cows.

Much research has been done to determine reasons for reduced availability of P from cereal grains. This has centered around biological availability of P bound to phytate (Figure 2.1). Amount of phytate in various feed grains has been measured. McCance and Widdowson (1944) reported whole wheat, oats, barley, and rye contained between 19 and 26% of DM as P bound to phytate. O'Dell et al. (1972) dissected kernels of corn, wheat, and rice. Whole kernels and fractions were analyzed for total P and P bound to phytate. Phosphorus bound to phytate was 83, 76, and 81% of total P for corn, wheat, and rice. Lolas et al. (1976) subsequently reported on the percentage of total P bound to phytate for barley, oats, soybeans, and wheat. Mean value of 15 cultivars of soybeans was 53.4% of total P bound to phytate. Total P bound to phytate was 61.5 (19 cultivars), 67.7 (19 cultivars) and 71.7% (38 cultivars) for whole oats, barley, and wheat. Wheat mill bran had more P bound to phytate (87.1%) than did whole wheat (71.7%). In cereal grains P bound to phytate serves as a source of P and inositol at germination.

Phosphorus bound to phytate is not available for biological processes until phytase hydrolyzes PO_4 from the inositol ring. Two sources of phytase are found in ruminants. The first source is produced by mold or bacteria and is found on cereal grains (Cannon, 1940, McCance and Widdowson, 1944). The second source of phytase is that produced by microorganisms within the digestive tract (Nelson et al., 1976, Rann et al., 1956).

Cereal grains contain phytase in varying quantities. Native phytase in cereal grains hydrolyzed P bound to phytate when exposed to water at 50 C and adjusted to pH 4.5 by 2N HCl (McCance and Widdowson,

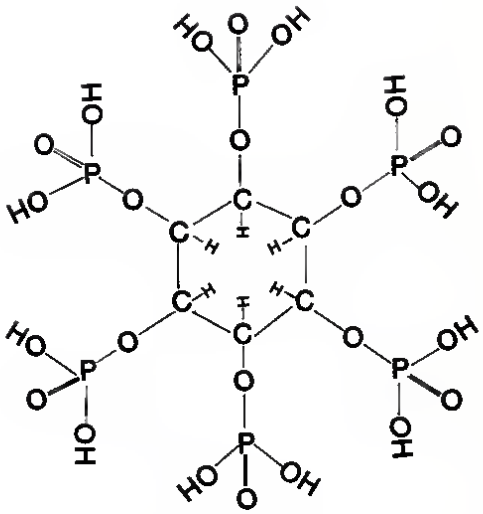


Figure 2-1. Molecular structure of Phytic acid. P, O, C, and H represent phosphorus, oxygen, carbon, and hydrogen

1944) They determined the amount of time required for hydrolysis of 50% of the P bound to phytate. This ranged from less than 1 h (barley, rye, and wheat) to greater than 10 h (oats, green or kilned). Under acidic conditions P bound to phytate was hydrolyzed to varying extents by native phytase.

Tillman and Brethour (1958b) compared calcium phytate and calcium carbonate monocalcium phosphate as sources of P using 12, 18-mo-old wethers. Two groups of six wethers were used to evaluate apparent and true digestibilities of each source of P and hydrolysis of P bound to phytate. A basal diet contributed .64 g of P/d and supplemental P provided 1.5 g/d. True digestibility of P was determined by I.V. infusion of ^{32}P. Apparent and true digestibilities of total P were -12.6 and 62.4% (calcium phytate) and -12.6 and 69.6% (calcium carbonate monocalcium phosphate). Wethers fed calcium phytate consumed .33 g of P bound to phytate from their diet and 1.5 g of P bound to phytate from the supplement. Digestibility of P bound to phytate was 91.8% and was similar for both sources of P. It was not determined where in the digestive tract P was hydrolyzed from the inositol ring.

Reid et al. (1947) reported that P bound to phytate was almost completely hydrolyzed in the rumen of sheep. They suggested this resulted from action of microbial enzymes. Raun et al. (1956) determined solubility of P bound to phytate when incubated in vitro with rumen microorganisms. They measured cellulose digestion during 24 to 72 h incubations in a series of small fermentation tubes to which graded amounts of Ca phytate or laboratory standard of P_i were added according to the method of Anderson et al. (1956). Rumen fluid was harvested from a steer fed a high roughage diet. Increase in concentration of P_i in

solution after incubation with viable microorganisms was assumed to represent the amount of P bound to phytate hydrolyzed by phytase produced by the microorganisms. As much as 27% of P bound to phytate was hydrolyzed by microorganisms. Solubility of P bound to phytate was near 100% when compared to results obtained when corresponding concentrations of P_i from laboratory standard ware used. This procedure may have underestimated hydrolysis of P bound to phytate since the amount of P hydrolyzed and then absorbed by the growing bacterial cells was not quantified. A more precise estimate of hydrolysis of phytate would result if actual quantity of P bound to phytate was measured along with changes in concentration of P_i. Additionally, rumen fluid was obtained from a steer consuming a high roughage diet. Greater hydrolysis may have occurred if rumen fluid had been removed from an animal consuming large quantities of concentrates, which likely would have contained greater quantities of phytase producing microorganisms.

Clark et al (1986) fed 30 Holstein cows diets of 50% roughage and 50% concentrate (DM basis) which were supplemented with inorganic sources of Ca during the first 18 wk of lactation. Apparent digestibility of P bound to phytate was 98%. This indicated P in phytate was available for absorption by the animal.

In vitro studies (Barth and Hansard, 1962) compared the availability of P to rumen microorganisms from phytate and several inorganic sources. The utilization of P bound to phytate decreased from 100% when Ca:P was 2:1 to only 67% when Ca:P was 8:1. They concluded that at wider Ca:P ratios the decreased utilization of P bound to phytate was related more directly to chemical effect than to inhibition of phytase production or action. Thus it appears that carefni

consideration of concentrations of Ca and P as well as Ca:P ratios are necessary when formulating diets high in phytate for ruminants.

When phytate is present in diets availability of cations must be considered. Phosphorus bound to phytate can bind polyvalent cations (Ca, Mg, Zn, Cu, Mn, Fe) and render them unavailable for absorption. Greater concern for availability of polyvalent cations exists for nonruminants, as they lack a fermentation compartment prior to the small intestine (Cruickshank et al., 1945, Davies and Olpin, 1979; Gillis et al., 1957, Graf and Eaton, 1984; Hintz et al , 1973; Nelson, 1967; Pointillart et al., 1984; Pointillart et al , 1986, Taylor and Coleman, 1979).

Another naturally occurring chelator of minerals is oxalate. Concentration of oxalate in tropical grasses and the effect of oxalate on availability of minerals has been studied. Blaney et al. (1982) reported oxalate content of tropical grasses reduced Ca availability by 20% but had no effect on availability of P or Mg.

Until recently few investigations critically evaluated availability of P in feedstuffs fed commonly to ruminants. Field et al. (1984) used four sets of 18-mo-old chimera derived triplet sheep to assess animal and dietary variation in the absorption and metabolism of P. The basal diet consisted of sugar beets and oat hulls. Addition of 1 of 12 feedstuffs at 10 to 30% of total diet constituted treatments. Feedstuffs examined included feeds high in content of protein (fishmeal, soybean meal, extracted rapeseed, rice bran, and corn gluten), two cereal grains (barley and wheat), and sources of roughage (ryegrass, clover, grass and lucerne nuts). Sheep consumed from 1.22 (barley) to 2.85 (rice bran) g of P/d. Protein and cereal diets were assigned and

consumed prior to roughage diets to minimize dietary induced changes in the ecology of the rumen. Absorption of dietary P was measured by specific activity of ^{32}P in excreta and plasma after i.v. injection of ^{32}P. Diets with a high concentration of protein (fishmeal, soybean meal, extracted rapeseed, rice bran, and corn gluten) had greater efficiencies of absorption of P (84, 81, 74, 87, and 78%) than did diets which contained greater concentrations of cereal (barley, 75% and wheat, 77%). Apparent digestibilities of DM and organic matter for diets with high concentrations of protein or cereal ranged from 89 to 91% and from 59 to 67%. Efficiency of absorption was not related to quantity of P consumed. Absorption of P was not reported for diets with additional roughage. Apparent digestibilities were reported for diets with added roughage and ranged from 86 to 88% (DM) and from 55 to 75% (organic matter). For the feedstuffs analyzed at least 70% of P was absorbed. It is not known how much of the remaining P was available and not absorbed.

A. Inorganic Phosphorus. The second form of P fed is inorganic. Various inorganic forms are present naturally to a small extent in feedstuffs, but to a greater extent they are fed as supplements. It is clear that all sources of P_i are not equal. Calcium meta- and pyrophosphates may be formed from rock phosphate during the thermal defluorinating process. The extent of their formation depends on temperature and duration of time exposed to high temperature during defluorination. These sources are not as available to the animal as other sources of P_i. Most research to evaluate biological availability of sources of P_i and to compare various sources of P_i for ruminants has been done using sheep.

Lofgreen (1960) utilized a radioisotope dilution technique to eliminate endogenous phosphate interference in order to determine the true digestibility of several P_i supplements and calcium phytate. The true digestibility of dicalcium phosphate was assigned a value of 100% and other phosphate sources were compared on a relative basis. Digestibilities were 92%, bone meal; 28%, soft phosphate; and 66%, calcium phytate.

Tillman and Brethour (1958a) evaluated absorption and retention of sodium meta- ($NaPO_3$), ortho- ($NaH_2PO_4 \cdot H_2O$), and pyro- phosphates ($Na_2H_2P_2O_7$) by an isotope dilution technique. Excretion of fecal endogenous (metabolic) P was greater when sodium metaphosphate was fed compared to excretion of endogenous P when sodium ortho- or pyro phosphate were fed (1.22 vs .97 and .98 g/d of total P excreted). This implied P from sodium metaphosphate was either more soluble within the GIT or was absorbed from the GIT with a greater efficiency. Apparent digestibilities of sodium ortho- and pyro- phosphate were 32.3 and 34.7%, but was only 16.8% for sodium metaphosphate. The percent of fecal P of endogenous origin was greater for sodium meta- than for sodium ortho-, or pyro- phosphate (21.2% vs 36.5 or 37.9%). Sodium metaphosphate was absorbed from the GIT and subsequently excreted into feces. True digestibilities of sodium ortho-, meta-, and pyro-phosphates were similar (98.9, 87.4, and 90.4%).

Ammerman et al. (1957) reported increased concentration of P in plasma of lambs when they consumed calcium ortho-($Ca(H_2PO_4)_2 \cdot H_2O$), meta-($Ca(PO_3)_2$), and pyro- phosphate ($Ca_2P_2O_7$). After having been on a P depleted feed for 30 d, true absorption of P from calcium ortho-, meta-, and pyro- phosphates were 40, 10, and -22%. The negative true

digestibility for calcium pyrophosphate indicated net excretion of endogenous P into feces. If calcium pyrophosphate was unavailable and not responsible for stimulating excessive excretion of P by lambs, the average true absorption should be zero instead of -22%. Correcting true absorptions for assumed unavailability, true absorption values became 63, 34, and 0% for calcium ortho-, meta-, and pyro- phosphates. After 2 wk of consuming feed with supplemental P as calcium ortho-, meta-, or pyro- phosphate, concentration of P in plasma was elevated by 2.5, 1.2, and .7 mg %. Some absorption and ultimate excretion of pyrophosphate must have occurred. Animals had a reduced ability to use effectively sodium or calcium meta- and pyro- phosphate.

Fisher (1979) compared availability of four inorganic sources of P (dicalcium phosphate, monocalcium phosphate, monoammonium phosphate and monosodium phosphate) in yearling Holsteins (two heifers and two steers) using a 4 x 4 latin square design. Source of supplement had no apparent effect on concentrations of P, Ca, or Mg in plasma or on excretion of P in feces. The author concluded that for the supplements evaluated no differences existed in availability of P. Although intake and digestibility of DM were not influenced by source of P, apparent digestibility of acid detergent fiber (ADF) was greater when monosodium phosphate (59.2%) was included than for monoammonium phosphate (56.5%) ($P < .05$).

Chicco et al. (1965) used wethers to determine the relative biological availability of the P in meta- and pyro- phosphates with mono-(sodium) and di- valent (calcium) cations present in the phosphate. The five sources evaluated were sodium meta-, and pyro- phosphate, and calcium ortho-, meta-, and pyro- phosphate. Availability of P was

measured as apparent absorption, net retention, absorbed dose retained, and tissue deposition of ^{32}P. Apparent absorptions were 47.2% (calcium ortho-), 45.8% (sodium meta-), 38.8% (sodium pyro-), 33.0% (calcium meta-) and 25.7% (calcium pyro- phosphate). Sources of P were ranked similarly for net retention, absorbed dose retained, and tissue deposition of ^{32}P. For sources of P other than calcium pyrophosphate, 97% or more of absorbed dose was retained. For calcium pyrophosphate, 69% of absorbed dose was retained. When phosphate sources were compared to calcium orthophosphate (assigned a value of 100% apparent absorption) other phosphates were 97% (sodium meta-), 92% (sodium pyro-), 70% (calcium meta-), and 54% (calcium pyro- phosphate).

Chicco et al. (1965) also tested availability of their sources of P in vitro. In a P deficient environment calcium meta- and pyro-phosphates were less available (78% and 0%) than other sources of P (> 95%) for cellulose digestion. Overall, their data supported the findings that improper manufacturing processes and higher temperatures have a negative effect on biological availability of calcium meta-, and pyro- phosphates. Differences in availability of calcium meta- and pyro- phosphates in vivo and in vitro may depend upon the form of the crystalline modification which occurs during the defluorinating process.

Studies with beef cattle have focused on evaluation of commonly used supplements of P such as, reagent and feed grade dicalcium phosphates, bone meal, phosphoric acid, defluorinated phosphates and soft phosphate. Dicalcium phosphate was more available than soft phosphate (Arrington et al., 1963, Ammerman et al., 1965, Long et al., 1956, O'Donovan et al., 1965). Relative availabilities among other sources of P were less definite (varied in magnitude of response).

Apparent absorptions of soft phosphate for cattle were 17% (Long et al., 1956), 54% (Arrington et al., 1963), and 88% (Ammerman et al., 1965). Large variations in relative biological availability may have resulted from the animal's previous status of P nutrition, origin of P supplement, and refining procedures.

Although much of the original work dealing with the animal's requirement for P was conducted using dairy cattle, little work has been reported on the comparative biological value of various sources of P. Wise et al. (1961) used 50 calves to evaluate comparative value of sources of P. Calves started on the experiment at 7 to 8 wk of age and were fed assigned diets. Males were used in experiment I (56 d in duration) and females were used in experiment II (99 d in duration). The basal diet was formulated (DM basis) for .11% P (experiment I) and .085% P (experiment II). The four remaining treatments were formulated to .19% P (slightly deficient for age group) by addition of one of four P supplements to the basal diet (dicalcium phosphate, defluorinated phosphate, Curacao Island phosphate (low fluorine rock phosphate), or colloidal clay (soft phosphate)). Body growth, bone growth, bone ash, concentration of P in serum, and concentration of blood phosphatase were used as criteria of availability. Concentration of P in serum was the most sensitive and most reliable biological availability measure. Concentration of P_i in serum ranged from 4.49 to 6.97 mg % (experiment I) and from 2.93 to 5.26 mg % (experiment II). Lower concentrations of P_i in experiment II probably were due to longer duration of feeding a slightly P deficient diet (43 d longer than animals in experiment I). Relative order of response (high to low availability) in the two

experiments was dicalcium phosphate, defluorinated phosphate, Curacao island phosphate, and soft phosphate

Calcium:Phosphorus Ratio. Most research addressing Ca to P ratio (Ca:P) has been done using monogastrics, predominantly poultry and swine. Research with ruminants has been limited. Contrary to monogastrics, ruminants usually can tolerate wider diet Ca:P without decreased availability of P (Herst, 1986). It is accepted (NRC, 1989) that when all nutrients are adequate, dietary ratio of Ca:P can be greater than 2:1 for ruminants.

Lamb et al. (1934) fed dairy heifers diets with Ca:P from 3.7:1 to 6.5:1. Eleven heifers started the experiment between 18 and 30 mo of age. Heifers were assigned to one of two diets before calving, low (.20%) or high (.41%) concentration of P and they were maintained on their assigned diet through first lactation. All other nutrients in these diets were adequate. Heifers consuming a diet low (deficient) in P utilized P more efficiently than heifers consuming a diet high (adequate) in P. Dowe et al. (1957) evaluated effects of feeding excessive Ca to growing beef calves when there were adequate concentrations of all other nutrients in the feed, including P. Forty (year 1) and 36 (year 2) 8-mo-old steers were grouped into one of four groups. Calcium to P ratios were 1.4:1, 4.4:1, 9.1 and 13.7 to 1 for the four groups. Average daily gains for 1.4:1 and 4.4:1 ratios were .60 and .59 kg. Reduced daily gains (.51 and .50 kg) occurred in steers fed the wider ratios. Improved efficiency of absorption of P was consistent with conservation of P when fed a diet deficient in P. Improved efficiencies of absorption of P also have been reported in pigs fed diets low in concentration of P (Fox and Care, 1978, Fox et al., 1978).

Since no adverse effects of Ca:P were measured, Dowe et al. (1957) concluded a critical Ca:P for growing calves must fall between 6.5:1 and 9.0:1.

Ricketts et al. (1970) fed Holstein steers from 13 to 37 wk of age diets varying in Ca:P ratio. Feed intake, average daily gain, growth (wither height, distance from floor to barrel, heart girth, and length) and concentration of P in serum were recorded. Steers fed Ca:P ratio of 9:1 consumed less feed and gained less weight than steers consuming a diet of Ca:P of 1:1 or 4:1. There were no differences in feed efficiencies (19.6, 18.8, 18.4% of feed consumed converted to body weight) or growth of animals fed diets having Ca:P ratios of 1:1, 4:1, or 8:1. Concentration of P in serum was greater in steers consuming 1:1 Ca:P diet (5.0 mg %) than other steers (3.4, and 3.4 mg %). These differences probably had no biological significance.

There may be two explanations as to why ruminants can tolerate wider Ca:P ratios than nonruminants. More acidic conditions are present in the small intestine, the primary site of absorption of P in ruminants. This should reduce the amount of P that would precipitate out as insoluble tricalcium phosphate. Also, the large quantity of P recycled into the GIT via saliva by the ruminant will alter dietary Ca:P ratio drastically.

Absorption

Availability, absorption, and retention of dietary and endogenous P are key regulators of P in body tissues. Absorption and secretion of P out of and into the GIT have been studied. Studies in rats, chicks, and dogs suggest absorption of P in intestines occurs via simple diffusion (Crenar, 1969, McHardy and Parsons, 1956, Morgan, 1969). In ruminants a

facilitated transport process involving a carrier system has been suggested (Care et al., 1990)

Phosphorus is similar to other nutrients When quantities fed are above that required, efficiency of absorption of P is less than when it is fed in quantities less than required Data reported in the literature pertaining to absorption of a nutrient are most applicable to the specific animals used (age, sex, breed) in combination with the specific diet constituents Simple balance trials in dairy cattle and sheep (Hibbs and Conrad, 1983; Lomba et al., 1969; Towns et al., 1979) were used to determine apparent digestibility (net absorption) of P

A more sophisticated technique using ^{32}P has been utilized to determine endogenous or metabolic fecal P Once specific activities of ^{32}P of blood and digesta were determined, differentiations could be made between P absorbed and reentered into the digestive tract and P not absorbed Use of ^{32}P allowed calculation of endogenous P and also permitted calculation of rate of secretion of body P into the GIT (Kleiber et al , 1950) Currently, studies utilizing this technique are limited because of regulations pertaining to use of ^{32}P (complete collection and storage of excreta for 10 half-lives--140 d). To reduce quantity of excreta, most studies in ruminants using ^{32}P have used nonlactating sheep, with very few studies using lactating dairy cows Many additional studies to determine absorption of P have been carried out in nonruminants, primarily chicks.

Cannulation of the GIT or sacrificing the animals and dissection of the GIT provided researchers an opportunity to study individual sites of absorption of P (Chandler and Cragle, 1962) They reported that net addition of P to contents of the GIT occurred prior to or in the rumen,

in the omasum and duodenum. Net absorption of P from the GIT occurred in the ileum and jejunum. The primary site of absorption of P is the upper small intestine in sheep (Ben-Ghedalia et al., 1975; Kay and Pfeffer, 1970; Pfeffer et al., 1979) and cattle (Bertani et al., 1976). In cattle, absorption of P from the omasum has been documented (Banks and Smith, 1984; Smith and Edrise, 1978).

Scharrer (1985) evaluated net absorption of P in lambs (< 13 wk of age) fed milk or solid diets. Absorption of P was determined in anaesthetized lambs with ligated intestinal segments filled with electrolyte solution. In lambs 1- and 4-wk-old, greatest absorption of P was in the mid jejunum with additional absorption in the proximal jejunum and colon and least amount absorbed in the distal jejunum. In 10- to 13-wk-old lambs absorption of P in the proximal jejunum and colon was minimal with greatest absorption in the mid and distal jejunum for both milk or hay and concentrate fed lambs. In young lambs the proximal and mid jejunum were more important for absorption of P than was the distal jejunum. Yet, in older lambs greater absorption of P was in the mid and distal jejunum. This serves to reinforce the fact that results from one experiment can not be extrapolated to an entire species or population. Diet and age of experimental animals are variables that must be considered.

Yang and Thomas (1965) quantified absorption and secretion of P by young calves fed low fiber (6.5%) or high fiber (26.6%) diets. Lignin and Cr_2O_3 were used as markers to determine differences in net absorption or secretion of nutrients measured in various compartments of the GIT. Use of lignin or Cr_2O_3 gave similar results for digestibilities. The digesta in a given compartment was considered to

be feed for the next posterior compartment. The digesta in the second compartment would be substituted for feces in a traditional (feed/feces)*(feces/feed) ratio technique (Kane et al., 1953). Samples of contents in the GIT were obtained from the reticulorumen, omasum, abomasum, upper small intestine, lower small intestine, large intestine, cecum, and rectum. Net absorption of P occurred in the reticulorumen, omasum and abomasum. Phosphorus was secreted into the upper small intestine and absorbed from the lower small intestine, large intestine, and cecum. Concentration of fiber in diet did not affect absorption of P in the various compartments.

Towns et al. (1978) fed four wethers a roughage diet. Wethers consumed 1.08 g of P and 4.01 g of Ca daily. After a 2 wk period of adjustment, 1.5 g of P/d (isotonic Na_2HPO_4/NaH_2PO_4 at pH 7.4) were infused i.v. (387 ml/d during 18 h). Within 1 d after infusions began, concentration of P in serum increased from 4.2 to 13.0 mg %. Concentration of P in serum stabilized at 5.8 mg % within 5 d after infusions began. Daily secretions of P in saliva were calculated assuming 10-fold greater concentration of P in saliva than in plasma. This assumption was valid for concentrations of P in plasma up to 6 mg % based on work by Tomas et al. (1967). Estimated true intestinal absorption of P was determined to account for P balances observed (81% preinfusion; 66% during infusion). If true absorption remained unchanged during infusion of P it would have been necessary to postulate another mechanism for the increased secretion of salivary P regardless of concentration of P in plasma. It was theorized that increased secretion of P by salivary glands combined with decreased absorption of P in the intestines accounted for observed changes in balance of P.

Schneider et al. (1982) infused sheep i.v. with isotonic saline or isotonic NaH_2PO_4/Na_2HPO_4 (pH 7.4) to provide 0 or 1.5 to 2 g/d additional P. The purpose of the experiment was to determine if absorption of P from the GIT or secretion of P in saliva was altered by i.v. infusion of P. Control animals received i.v. infusion of saline. Radiolabelled ^{32}P was injected i.v. or abomasally and plasma samples from jugular vein were taken at 2-minute intervals for 30-min and then at increasing intervals for 5 to 7 d. Injection of ^{32}P i.v. into sheep infused with P did not alter the pattern of disappearance of ^{32}P from plasma. The subsequent appearance of ^{32}P in feces was increased. These seemingly contradictory results probably were due to fluctuations in concentrations of P in plasma which in turn were influenced by outflow of P from saliva and by inflow of P from the GIT. Abomasal injection of ^{32}P resulted in ^{32}P appearing more slowly in plasma and at a lower peak for sheep infused with P vs saline. Cumulative rates of absorption of P (% of isotope absorbed in 60 min) were greatest during i.v. infusion of saline (33 to 58%) and least during i.v. infusion of P (18 to 43%). This confirmed the theory of Towns et al. (1978) that absorption from the GIT was reduced when P was infused i.v.

An important result from Schneider et al. (1982) was the low rate of excretion of ^{32}P in feces after abomasal injection of ^{32}P. Absorption of P during i.v. infusion of saline was about 85% and this decreased to 73% during i.v. infusion of P. This decrease was not related to changes in concentration of P in abomasal fluid. The ratio of concentration of P in abomasum to concentration of P in blood decreased during infusion of P. This should have reduced passive diffusion of P from gut to blood. Since the change in ratio of concentration was not uniform and

absorption decreased in all sheep, Schneider et al (1982) concluded that both active and passive forces were involved in regulation of absorption of P.

Young et al. (1966) reported that sheep fed diets deficient in P for 134 or 142 d showed adaptation in their absorption of P. After sheep had consumed a diet deficient in P for 4 d there was a decrease in concentration of P in serum. The authors concluded that rate of secretion of P into the digestive tract might be regulated by concentration of P in serum.

It is quite possible that hypophosphatemia resulting from ingestion of a diet low in concentration of P acted two-fold to maximize utilization of P. Firstly, reduced concentration of dietary P would increase efficiency of intestinal absorption of dietary and secreted P. Secondly, reduced concentration of P in diet would reduce secretion of P into the GIT via saliva.

Secretion, Excretion, and Retention

The origin of P in the GIT can be either exogenous or endogenous. Exogenous P is unabsorbed P which enters the digestive tract, passes through the animal and is excreted in feces. Conversely, endogenous P is absorbed into the body and is returned to intestinal contents. This can occur by diffusion from blood or interstitial tissue fluid, as part of secretions such as saliva, bile, or other digestive juices, as a component of cells or cell fragments sloughed off from the intestinal lining, or contained in phagocytes (Kleiber et al., 1951). Fecal P from microorganisms is exogenous when taken up from unabsorbed compounds, or endogenous when its origin can be traced to saliva, digestive juices, or other metabolic sources.

Since excretion of P in feces is the principle pathway of excretion, metabolic fecal P represents a major part of total loss of P from the body. Lofgreen et al. (1952a) fed radiolabelled (^{32}P) casein as a tracer to study digestion and absorption of casein by calves. Four calves were fed a purified diet containing unlabelled casein from 2 to 3 d of age until radiolabelled casein was fed at week seven. Metabolic fecal P was 62, 70, 74, and 75% of total fecal P in the four calves. Apparent and true digestibilities of P from casein were 74 and 93%.

Methods to decrease rate of secretion of saliva (fasting or infusion of liquid diet) can divert part of endogenous excretion of P from feces to urine (Tomas, 1974a, 1974b). This would make P unavailable for reabsorption from the small intestine.

The ability to calculate endogenous fecal P permits determination of 'true' digestibility of P in feed (the degree to which content of P in feed can be absorbed by the animal). True digestibility of P is calculated as: [(intake of P (g) from feed minus exogenous P (g) in feces) / intake of P (g) from feed]*100. Apparent digestibility is calculated as: [(intake of P (g) from feed - excreted P (g) in feces) / intake of P (g) in feed)*100. The latter method is a valid way to estimate 'true' digestibility only when contribution of endogenous P to total fecal P is small and excretion of P in urine is negligible. Kleiber and coworkers (1951) reported that excretion of endogenous P in feces exceeded excretion of exogenous P in feces of cows. Hence, use of apparent instead of 'true' digestibility gives a much reduced estimate of true digestibility.

In cattle, as a result of high endogenous contribution of P to the GIT, apparent absorption is less than true absorption. Since cattle

excrete 90 to 95% of P via feces (Forbes et al., 1922, Hibbs and Conrad, 1983; Kleiber et al., 1951) and P is recycled via saliva, it is important to quantify excretion of endogenous P in feces for a more precise estimate of 'true' digestibility. In man, calculation of endogenous P is less important as excretion of P in urine may exceed excretion of P in feces (Clark, 1925)

Towns et al. (1978) used four wethers to quantify excretion of P and effects of i.v. infusion of P on excretion of P. Wethers were fed oat and lucerne hay (1 08 g of P/d) for 5 wk. After 3 wk, 1.5 g of P/d were infused i.v. for 18 h daily for 11 d. All excreta were collected during week 1, on the 11th d of infusion, and 1-wk-postinfusion. Three of four sheep had no change in excretion of P in urine (10 ± 3 mg/d) from the week preinfusion through the week postinfusion. In the fourth sheep excretion of P in urine increased from 9 ± 1 mg/d (preinfusion) to 367 ± 119 mg/d (infusion) and then decreased to 45 ± 60 mg/d (postinfusion). Large animal variation existed in excretion of P in urine. Excretion of P via urine within and among sheep was variable. Excretion of P in feces increased from 930 mg/d (preinfusion) to 2100 mg/d (infusion). The primary pathway of excretion of P was in feces and a small but variable amount of P was excreted in urine.

Scott and Beastall (1978) used seven ruminally fistulated mature sheep to determine effects of i.v. infusion of P on secretion of P into saliva. Sheep were fed 900 g of pelleted grass (DM) given in two equal portions daily. About 18 h prior to i.v. infusion sheep were anaesthetized and a parotid salivary duct was cannuleted. Saliva was collected and returned through the rumen cannula. A total of 1.6 g of Na_2HPO_4 was infused i.v. during six 30-min infusions. Intravenous

infusion of P_i increased concentration of and amount of P_i secreted in saliva to about twice that determined during the control periods. No consistent effect on salivary flow was observed. Intravenous infusion of P increased endogenous secretion of P via salive but did not affect rate of flow of saliva.

Total quantity of P in the animal may be partitioned into a series of compartments or pools. Knowledge of these pools aids in understanding absorption, secretion, excretion, and retention. Characterization of each pool requires knowledge of exchange rates with other pools, and its own capacity. Early work by Garton (1951) characterized distribution of P_i in the stomach of sheep. He found that the concentration of P_i in strained liquor from the rumen or abomasum were the same (30 to 40 mg %). Phillipson et al. (1949) quantified dilution of omasal contents by gastric juice of sheep (1 part omasal contents to 1 to 2 parts gastric juice). Garton (1951) cited a review by Favilli in which he concluded that the omasum does not function merely to press and squeeze food, but also to effect comminution and to absorb water. Since concentration of P in the rumen and abomasum were similar, no net absorption or secretion of P occurred. Any absorption of P prior to the abomasum was negated by secretion of gastric juices in the abomasum.

Schneider et al. (1987) quantified excretion of P in mature sheep by compartmental analysis. Sheep were prepared with reentrant parotid cannulas, or cannulas in the rumen, abomasum, duodenum, jejunum, and colon. Compartments included blood, soft tissue, bone, rumen (saliva), abomasum and upper small intestine (digestive juices), lower small intestine, and cecum-colon. Sheep were fed alfalfa and oat hay (50:50)

(3.75 g of Ca and 1.4 g of P/d) or infused with liquid diet into the abomasum (soybean protein, vegetable oil, vitamin and mineral mixture formulated to provide 2.0 g of Ca and 1.9 g of P/d). Dietary treatments (hay, hay 50% and liquid 50%, or all liquid diet) and infusion of ^{32}P were arranged such that each compartment within the digestive tract could be isolated. Rate constants for all compartments except urine were similar. Concentration of P in urine was greater for animals infused with liquid diet than for animals consuming hay diets. Secretion of endogenous P was calculated from excretion of P in feces when liquid diet was infused. Under these conditions, salivation was reduced and P in feces was derived mainly from digestive juices. Digestive juice secretion into the GIT probably was decreased due to lack of solid matter in the GIT. Concentration of P in plasma and excretion of P in urine increased in animals infused with the liquid diet. After discontinuing the infusion of the liquid diet, sheep gradually increased intake of roughage and consequently increased rate of secretion of saliva.

Lofgreen et al. (1952b) reported data of ^{32}P excretion into the GIT of young calves (45- to 67-d-old). Two catheters were inserted into jugular veins. Calves were infused i.v. with ^{32}P every 20-min for 24 to 34 h to maintain a constant concentration of ^{32}P in plasma. Two calves were slaughtered after 24 h of ^{32}P infusion. Samples of tissues and contents of the rumen, omasum, abomasum, jejunum, cecum, colon, and rectum were taken. Concentrations of total P and ^{32}P were determined. High concentrations of ^{32}P were observed in rumen, omasum, abomasum, and jejunum contents. The authors believed these compartments were important in excretion of endogenous P. They stated that the high

activity of ^{32}P of ruminal and omasal contents indicated P readily passed from blood into these compartments. They postulated that the rumen wall was a passive barrier through which P_i may pass in either direction, depending upon the conditions existing in blood and the GIT. Alternately, their findings could have been attributed partially, if not completely, to flow of ^{32}P in saliva into the rumen.

Many researchers have quantified secretion of P from endogenous sources into the GIT. Secretion of ^{32}P into contents of the GIT in sheep differed from that found in nonruminants. Based on estimates of concentration of P in saliva (.81 g/l) by McDougall (1948), 6 l of saliva would need to pass into the rumen daily to achieve the reported concentration of P in rumen contents. This flow rate was greater than the 4 to 5 l/d observed by Watson (1933). Smith et al. (1955), concluded that half of endogenous P entered the rumen from some source other than saliva. They postulated that there was passive entry of P through rumen wall (ruminal epithelium is devoid of secretory glands). Work by Scarlsbrick and Ewer (1951) and Parthasarathy et al. (1952) indicated that rumen epithelia was permeable to P_i and that P_i did transfer across it. They concluded that net absorption of P_i from the rumen over extended time periods was minimal. However, at any particular instant there may be a substantial movement of P_i into or out of blood transversing the rumen wall.

Smith et al. (1955) used 15 lambs 1 to 10 mo of age to determine secretory compartments of the GIT. Sheep were injected I.V. with ^{32}P and returned to their usual routine until time of slaughter, which varied from 5 to 72 h postinjection. After sacrifice, samples of contents and tissue were collected from the rumen, omasum, abomasum,

small intestine (after entry of bile and pancreatic juice), cecum, colon, and rectum. Concentration of total P and ^{32}P were determined as well as DM content of digesta. In 1-mo-old lambs secretion of ^{32}P into the GIT was small with greatest activity found in contents from the rumen. Low flow of saliva in nursing lambs was probably responsible for low concentrations of ^{32}P in the rumen. In the 10-mo-old lambs the greatest specific activity was in contents from the omasum or abomasum. These authors concluded that an active process of secretion of P or selective resorption of water occurred in the omasum or abomasum. It is difficult to understand why they found greater concentrations of ^{32}P in the omasum and abomasum than in the rumen in 10-mo-old lambs. Content of DM increased in compartments from the rumen through the abomasum in all aged lambs. There was an increased content of moisture in the small intestine due to secretions of bile and pancreatic juices. Content of DM increased posterior to the small intestine. This trend agreed with previous reports of decreased water content and concentration of P of tissues posterior to the upper small intestine (Garton, 1951, Lofgreen et al., 1952b).

As sheep and cows are both ruminants, similarities exist between their digestive tracts. Comparisons of these species has indicated that relative capacity of forestomach compartments and epithelial lining exhibit structural differences (Sisson and Grossman, 1953). The quantity of volatile fatty acids (VFA) produced in the rumen of cattle, per unit body weight, was two-times greater than that in sheep (Elsden et al., 1946).

No research had compared secretion of P into the GIT in sheep and cattle. However, Smith et al. (1956) applied their technique of

injection of ^{32}P i.v. in sheep (Smith et al., 1955) to studies with dairy cattle (1956). They suggested that there were differences between sheep and cattle in secretion of P_i (a major buffering agent within the rumen) into the GIT. Location within the GIT of ^{32}P injected through the jugular vein was evaluated up to 72 h postinjection in calves and aged cows. Water content of GIT tissues and their contents were similar in both groups of animals. Excluding rumen epithelia, concentrations of ^{32}P in tissues of the GIT were greater in calves than cows. Concentrations of P in digesta were greater in cows than calves, but concentrations in rumen epithelia and rumen contents were opposite those found in the remaining sections of the GIT. Quantity of P in rumen contents, per kilogram of body weight, was 30% greater in dairy cattle than sheep.

In ruminants, salivary glands play a key role in homeostasis of P (Clark et al., 1973). Inflow of 5 to 10 g of P/d in saliva has been reported (Bruce et al., 1966, Kay, 1966; Pfeffer et al., 1970; Scott et al., 1984a, 1984b, 1985, Tomas et al., 1967). This was greater than the intake of 2 g of P/d. Phosphorus in saliva accounts for a large quantity of P secreted into the GIT. Saliva is a major component of turnover of P in ruminants.

In cattle, saliva can account for more than 70% of moisture entering the reticulorumen. This value excluded consumption of water (Bailey, 1961a, 1961b, Bailey and Balch, 1961a, 1961b). Bailey (1961a) determined rate of secretion of mixed saliva during eating and rumination and then estimated magnitude of total daily secretion of saliva. Various feedstuffs (mangels, dairy cubes, fresh grass, dried grass, silage, and hay) were evaluated in a total of five cows. Rate of

consumption of feed was inversely proportional to fiber content, increased quantity of fiber was associated with decreased intake per unit time. Cows fed more fibrous feeds had increased production and secretion of saliva than did cows fed less fibrous feeds. One cow secreted 1.15 g of saliva/g mangels consumed but she secreted 3.82 g of saliva/g hay consumed. There was a tendency for the amount of saliva secreted for each unit of more fibrous feed to decline throughout a meal and to increase again towards the end of the meal. Differences in content of DM or other feedstuff characteristics did not appear to affect secretion of saliva. The amount of saliva secreted for a given weight of feedstuff eaten depended on rate of secretion of saliva and rate of swallowing of food. Rate of swallowing was altered directly by occurrences of swallowing or indirectly by changing amount of food per bolus.

Bailey (1961a, 1961b) reported that secretion of saliva was 229 ml/min during eating; this was two- to four- times greater than secretion at rest. Rate of swallowing was monitored during eating and increased from less than 3 boluses/min (< 100 g food/min) to more than 5 boluses/min (> 350 g food/min). Frequency of swallowing had less effect on secretion of saliva than did size of bolus. Size of bolus increased from 15 to 38 g when feed was eaten slowly to 66 to 102 g when feed was eaten rapidly. Variations in rate of eating appeared to be of greater importance than differences in the rate of secretion of saliva to estimate flow of saliva. Estimated amount of fluid added to rumen ranged from 123 to 226 l/d with 98 to 130 l/d being of salivary origin.

Phosphorus is secreted into the GIT in abomasal juices (< .2 g/d, Barton, 1951; Kay, 1966) and is a constituent of both bile and

pancreatic juices. In sheep, no net absorption of P occurred in stomach compartments (Garton, 1951; Scarisbrick and Ewer, 1951, Parthasarathy et al., 1952) or in the large intestine (Bruce et al., 1966, Kay and Pfeffer, 1970). Contradictory findings were reported by Smith et al. (1955). They concluded that more P reached the rumen of sheep through sources other than diet and saliva. Their conclusion was based on estimated intakes and specific activities of plasma and rumen contents determined by specific activity of ^{32}P. Furthermore, they estimated concentration of P in saliva and assumed production of saliva was 56 l/d. The estimate of saliva production was conservative compared to other estimates (Bailey, 1961a, 1961b). This could account for the conservative estimate of contribution of salivary P to rumen P. Parthasarathy et al. (1952) made their determinations by direct measures of specific activities of ^{32}P in plasma and digestive contents. Their results showed no net absorption of P in stomach compartments and resulted from direct measurements and not from undocumented assumptions.

Rumen and duodenal contents of P_i averaged 35 mg % and 37 mg % (Garton, 1951). Secretion of P_i in gastric juice was negligible. He concluded that the rumen of sheep had no secretory glands per se, and that saliva provided much of the liquid medium of ruminal contents. McDougall (1948) calculated secretion of parotid saliva, the major component of mixed saliva, in sheep. Secretion ranged from 2 to 4 l/d. Phosphorus in mixed saliva primarily was P_i (37 to 72 mg %). Manas-Almendros et al. (1982) reported that there was a linear relationship between acutely induced changes in concentration of P in plasma and concentration of P in parotid saliva of sheep and goats.

Deficiency of Phosphorus

Nutrient deficiencies in the ruminant raises the question whether the animal is deficient in the nutrient or whether the rumen microorganisms are deficient in the nutrient. Diets should be formulated for ruminants considering the overall requirements of the animal, as well as the requirements by the rumen microbial population.

Deficiency of Phosphorus to Animal

To elicit signs of deficiency of P under experimental conditions, it has been necessary to feed ruminants rations that are low in P for an extended period of time. Animals in a stage of growth or early lactation were best suited to evoke signs of deficiency (Eckles et al., 1932).

Eckles et al. (1932) summarized signs of deficiency of P. These were bone chewing (pica), gnawing of wood, poor appetite (especially for roughage), stiffness in joints, and poor physical condition. Prolonged deficiency resulted in listlessness. Also, reduced feed intake, reduced efficiency of feed utilization, increased CO_2 production and O_2 consumption, decreased partial efficiency of energy utilization, and reduced efficiency of feed protein sparing for body protein were associated with deficiency of P (Braithwaite, 1976; Call et al., 1978; Carstairs et al., 1980; Kincaid et al., 1981; Kleiber et al., 1936; and Theiler et al., 1924). Deficiency of P has been implicated in decreased red cell glycolysis and therefore decreased ATP synthesis associated with bovine postparturient haemoglobinuria (Wang et al., 1985).

The one sign specific to deficiency of P was pica. Deficiency of P was prevalent in parts of Wisconsin, Minnesota, Colorado, Utah, Nevada, New Mexico, Florida, and California (Eckles et al., 1932). Deficiency

of P in animals occurred when animals grazed unimproved pastures or consumed roughages low in concentration of P.

Reduced concentrations of P in serum or plasma have been used to identify clinical deficiency of P (Becker et al , 1933; Eckles et al , 1932; Theiler and Green, 1932). This is the least invasive technique to determine P status. Reduced concentration of P in serum or plasma occurs after prolonged deficiency of P. Normal range for P in serum or plasma of lactating dairy cows is 3 to 9 mg %, yet in whole blood the concentration of P is from 35 to 45 mg % (Blood and Henderson, 1968). Since concentration of P in whole blood is 5- to 10- fold greater than concentration of P in serum or plasma it is important that proper care be taken to minimize hemolysis when retrieving serum or plasma samples. Phosphorus in bone is mobilized to some extent to maintain normal concentration in blood, but does not occur rapidly (Duncan, 1958). Concentration of P in blood may reflect only the rapid changes in concentration of dietary P, whereas, deficiency of P resulting from prolonged low levels of dietary P may be measured more precisely by concentration of P in bone. This is measured by bone breaking strength. Elevated concentration of P in blood occurred when animals were stressed or samples were obtained or handled improperly (hemolyzed). Therefore, apparently normal concentrations of P in blood may represent falsely adequate concentrations of P instead of representing adequate bone nutrition (Hemingway, 1967).

Bone breaking strength (Becker and Heal, 1930; Henderson and Weakley, 1930) has been used to determine effects of concentration of dietary Ca and P on bone forestion. This measure was affected more by concentration of dietary Ca than by changes in concentration of dietary

P. More recently, Little (1984) proposed that bone be used to determine reserves of body P in cattle. Two groups of six Hereford cattle were fed high P (HP) or low P (LP) diets. Four Shorthorns were fed low P and stressed physiologically by superimposing pregnancy and lactation on the LP ration (LPL). Actual concentrations of P in diets fed prior to slaughter were not provided. Animals were slaughtered (170 to 280 kg body weight) for bone analyses. Compact bone was quantified and osteoid tissue was determined. Concentrations of P in total fresh ribs were 5.41, 4.49, and 4.21% for HP, LP, and LPL groups. Level of significance for difference in P concentration in total fresh ribs among groups was not given, however the lowest value for HP treatment was 5.08% and the highest value for LP treatment was 4.71%. He suggested that concentration of P in total fresh rib be adopted as a criterion for determining reserves of P in cattle with 5% or more indicative of adequate reserves. This technique can be used in live animals by taking biopsies of rib bones. On the other hand bone breaking strength generally is used on bones recovered from dead animals as the entire bone (rib, femur, tibia, etc.) is needed for strength determination. Before Little's criterion can be adopted further research is needed to more precisely estimate an appropriate value and to determine if bone biopsies instead of entire rib analysis will be acceptable.

Belonje and Van den Berg (1983) compared effects of feeding three different concentrations of P to 15 6-mo-old sheep during a 98 d experiment. Dietary concentrations of P fed were .35, .47, and .64% and for Ca were 1.47, 1.85, and 1.37% (DM basis). All other nutrients were adequate. There were no differences in weight gain or in concentration of Ca or P in bones at slaughter. There was a tendency

for concentrations of Ca and P in bone to follow concentration of dietary Ca. The tendency for reduced concentration of P in bone with increased concentration of P in diets was explained by a reduced concentration of Ca in these rations. Concentration of P in bone was not indicative of dietary intake. There are two explanations for differences between cattle (Little, 1984) and sheep (Belonje and Van den Berg, 1983). First, the recommended concentrations of P and Ca in diets of sheep are .18 and .38% P and .20 to .82% Ca, the ration lowest in P was not deficient in P, yet the ration highest in P (.84%) was deficient in Ca (.37%). Additionally, the ration with high concentration of P had a Ca:P ratio of .58. Second, it was not known if the experiment was long enough to cause a deficiency in P, since previous status of P was not indicated.

Effects of deficiency of P on efficiency of reproduction have been inconsistent. Observations from areas where soils were deficient in P indicated that there was reduced efficiency of reproduction due to frequent anestrus and reduced conception rates (Brooks et al., 1984, Carson et al., 1978, Eckles et al., 1928, Sheehy, 1948, Theiler et al., 1928). In some cases, deficiency of P was confounded with poor quality and/or low quantity of feeds. This resulted in other nutrient deficiencies such as energy, protein, and minerals (Cohen, 1975). Other researchers reported that reduced intake of dietary P did not alter reproductive performance (Hignett and Hignett, 1952; Littlejohn and Lewis, 1980).

Eckles et al. (1935) evaluated reproductive performance of eight cows subjected to severe deficiency of P. They detected no change in frequency of estrous cycle of these cattle. As three of the animals

subjected to severe deficiency of P contracted Brucella abortus it was suggested that animals deficient in P were more susceptible to infection than herdmates. No control animals were evaluated. It was impossible to determine if susceptibility of animals to disease was a result of deficiency of P or merely a natural occurrence.

Researchers in Utah (Blake et al., 1976; Call et al., 1978; Call et al., 1987) evaluated effects of P intake on reproductive efficiency of beef cattle. A total of 96 Hereford heifers (two replicates of 48, one year apart) were fed a diet (DM basis) consisting of .14% P (66% NRC values) or .36% P (174% NRC values) (Call et al., 1978). Average daily weight gain (.44 kg/d) between 7 and 24-mo was similar in both groups. A greater percentage of heifers consuming .14% P diet tended to initiate ovarian activity by 1 yr of age than did heifers consuming .36% P diet (15 vs 6%). By 14 mo of age, a smaller percentage of the heifers consuming a diet containing .14% P were cycling than those consuming the ration higher in P (73 vs 81%). Concentrations of P in serum at 7 mo of age were 8.1 and 11 7 mg % and by 16 mo of age were 7.1 and 8.7 mg % for cows receiving the .14 and .36% P diets. The decrease in concentration of P in serum agreed with data reported (Nolier et al., 1977) that as animals mature, concentration of P in serum or plasma decreases. Concentrations of P in serum were not indicative of a deficiency of P.

Deficiency of Phosphorus to Rumen Microorganisms

In vitro studies can be used to determine requirements of microorganisms independent of the requirements of the host animal. Komisarczuk et al. (1987) utilized a continuous culture technique to study requirements of rumen microorganisms for P. Productivity of rumen microorganisms was measured by fermentation of carbohydrates, flow of

microbial nitrogen and digestion of organic matter. Each culture run of the experiment was 22 d in duration and was divided into four periods. The first period was 7 d and the remaining three periods were 5 d each. Each vessel contained 1 l of rumen fluid. A preliminary experiment determined the appropriate concentrations of P_i in vessels and characteristics of adequate growth of microorganisms and production of VFAs. In the preliminary experiment two culture vessels contained rumen contents (500 mg of P_i/l). One was infused continuously with artificial saliva containing of 480, 250, 100, and finally 0 mg of P_i/l. Concentrations of P in vessels were 411, 185, 54, and 2 mg of P_i/l. The other vessel served as a control and was infused continuously with artificial saliva containing 480 mg of P_i/l (411 mg of P_i/l). No differences in pH, total production of VFA, molar proportions of VFA, or concentration of ATP were apparent for the three higher concentrations of P_i compared to measurements for the control vessel. Since differences were evident between the lowest concentration of P_i and the control vessel, they evaluated lower concentrations of P_i (< 50 mg of P_i/l) during the main experiment. This corresponded to concentrations of less than 120 mg of P_i/l in the artificial saliva. For the main experiment the control vessel was infused with 120 mg of P_i/l during all four periods. The second vessel (treatment vessel) was infused with 120, 80, 40, and 0 mg of P_i/l which resulted in 48, 24, 4, and < 1 mg of P_i/l. Concentration of P in the treatment vessel was confounded with period. Sodium bicarbonate was added when P_i was removed from the second vessel to correct for loss of buffering capacity. Concentration of P_i did not affect number of protozoa. When concentration of P_i was < 1 mg/l, there was a greater production of ammonia nitrogen and an

elevated pH, compared to results from other periods. Average production of VFA in the control vessel was 173 mmol/d and did not differ from the treated vessel when concentration of P_i in the treated vessel was either 49 or 24 mg of P_i/l. At the lower concentrations of P_i (4 and < 1 mg of P_i/l), production of total VFAs was less (149 and 91 mmol/d). During the fourth period digestion of organic matter was depressed (9.1 vs 15.2 g/d), as was fermentation of carbohydrates (12.8 vs 19.3 g/d) and flow of microbial nitrogen (261 vs 485 mg/d) when treated vessel was compared to the control vessel (< 1 mg of P_i /l vs 120 mg of P_i/l). It is highly unlikely that the concentration of P in rumen liquor of lactating cattle would be reduced to concentrations which would cause deficiency for the microbial population. However, these results occurred *in vitro* and it is not known if they are an appropriate model for the more dynamic environment of the rumen.

Breves and Holler (1987) used five wether lambs, each with ruminal, reentrant duodenal, and T-shaped ileal cannulae. Objectives of the experiment were to determine effects of depletion and repletion of P on digestibility of DM and organic matter. Dietary intake of P varied between .91 and 1.04 g of P/d. Phosphorus (3.5 g/d) also was infused continuously into the rumen. Depletion of P was accomplished by omitting P from intraruminal infusion. After 3 wk of depletion of P, CrEDTA was infused into the rumen and excreta were collected for 6 d. Repletion occurred by twice daily infusion of 350 ml of P into the distal part of the duodenal cannula. This raised total supply of P to 4.1 g/d. Three wk after repletion, sampling was repeated for 6 d. Digestibilities of DM and organic matter were less in wethers depleted of P. Apparent digestibility of organic matter anterior to the proximal

duodenum was greater for wethers repleted with P (51.6%) than in wethers depleted of P (44.8%). No differences were detected for apparent digestibilities within the small intestine. Apparent digestibilities in the large intestine were greater for both DM and organic matter in wethers repleted with P (42.9 and 41.2%) than in wethers depleted of P (25.9 and 24.8%). Similar differences were found for apparent digestibilities within the total tract (79.9 and 78.8% compared to 69.3 and 70.7%). Lower digestibilities of organic matter anterior to the proximal duodenum and within the large intestine lend credibility to the idea that microbial activity in these compartments may be limited somewhat in vivo by reduced concentrations of P_i.

When sheep were fed a diet low in concentration of P but adequate in other nutrients, intake and digestibility of feed were reduced (Field et al., 1975, Milton et al., 1982, Sevilla and Ternouth, 1980). As P was supplemented to sheep intake of feed was increased and in some cases digestibility of feed also was increased (Milton et al., 1982, Sevilla and Ternouth, 1982). Milton and Ternouth (1984) found that as little as 30 to 80 mg of P_i/l was necessary for metabolism of microorganisms. Ternouth and Sevilla (1983) reported that intake of DM and concentration of P_i in plasma were correlated. This suggested that the effects of deficiency of P on intake and digestibility of feed were independent of each other. Reduced intake of feed may be due to impaired intracellular metabolism at some nondigestive site rather than to an impairment of ruminal digestion.

Milton and Ternouth (1985) tried to separate the digestive and nondigestive effects of deficiency of P on intake of feed. Four wethers with bilateral reentrant parotid duct catheters, ruminal fistulas, and

abomasal catheters were fed a pelleted semisynthetic diet high in Ca (7 g/d) and low in P (.5 g/d). Each wether received each of three treatments for 14 d. Treatments were as follows: 1) low ruminal and low blood P_i achieved by abomasal infusion of saline with parotid saliva returned to the mouth (LRLB); 2) high ruminal and high blood P_i achieved by abomasal infusion of P (1.3 g/d) with parotid saliva returned to the mouth (HRHB); and 3) low ruminal and high blood P_i achieved by abomasal infusion of P (4.7 g/d) with artificial saliva (no P) returned to the mouth (LRHB). Wethers in the HRHB treatment had twice the concentration of P_i in plasma than when they received the LRLB treatment. Concentration of P_i in plasma of LRLB group was 40% less than that of wethers in either the LRHB or HRHB treatments. There were no differences among treatments in digestibility of organic matter or retention time in the digestive tract compartments. Yet when wethers received LRLB treatment, intake of organic matter was reduced from 900 g/d to 592 g/d. This reduction did not occur when only concentrations of ruminal P_i were reduced (LRHB). Apparent digestibility of neutral detergent fiber (NDF) was reduced from either 48.9 or 47.1% (HRHB, LRLB) to 46.0% (LRHB). No depression in digestibility occurred with the LRHB treatment. The authors concluded that the reduction in intake of feed with the LRLB treatment was not associated with depression in digestion but was associated with some nondigestive tract disturbance.

Toxicity

Phosphorus toxicity problems in ruminants are associated generally with a relative excess of P to Ca (low Ca:P ratio). Urinary calculi (the formation of stones or calculi) is a result of inadequate Ca:P (Bushman et al., 1965). This metabolic problem results in obstruction

of the urethra by calculi which blocks excretion of urine. If unresolved, urinary calculi can result in bladder rupture which leads to abdominal distention, depression, and death from uremia (Blood and Henderson, 1968).

Excess P relative to Ca can result in bone disorders (osteomalacia, osteoporosis) (Bartter, 1964). High dietary P results in increased concentration of P in serum which results in decreased concentration of circulating Ca. Decreased concentration of circulating Ca stimulates parathyroid gland to increase concentration of Ca in serum by resorption of bone and to increase endogenous excretion of P. The net result is bone loss.

Superphosphate poisoning has been observed in late winter and spring in pregnant and lactating ewes under nutritional stress (O'Hare and Cordes, 1982). Signs of clinical problems include anorexia, thirst, diarrhea, weakness and incoordination. Signs are similar to those of hypocalcaemia but Ca therapy is ineffective. Death usually occurs within 48 h. Poisoning of sheep can be prevented by reducing or eliminating the frequency of grazing a short pasture topdressed with fertilizer.

Effect of Intake of Phosphorus on Excretion of Phosphorus

The majority of research in ruminants on the effect of dietary intake of P on excretion of P has utilized sheep and goats. Reduced costs of experiments are associated with sheep as consequences of lower intake of feed, lower production of excreta, smaller facilities, and better storing characteristics of excreta when ^{32}P is infused.

Increased loss of endogenous P was associated with increased intake of P in lambs fed at three deficient levels of P (Braithwaite, 1985).

these results indicated endogenous fecal loss of P was not maintained at a constant level for intake of P below maintenance.

A growth and metabolism study was conducted using 24 young lambs fed one of three diets containing .12, .15, and .29% P of diet DM in a complete ration (Preston and Pfander, 1964). These concentrations represented diets deficient, borderline, and adequate in P. Deficiency of P was established by reduced concentration of P_i in plasma from 6 to 3 mg %. After 7 wk, half of the wethers in each treatment group were injected subcutaneously with one dose of ^{32}P and all excreta were collected for 2 wk. Contents of P in feces, urine, and rumen samples were determined. The other animals from each group were fed an additional 17 d prior to injection with ^{32}P. The percentage of P_i in rumen fluid (endogenous source) tended to increase from 1.37 to 3.20 g as concentration of P in diet DM increased from .12 to .29%.

Scott et al. (1985) used three mature sheep fitted with ruminal and duodenal cannulae to measure secretion of P in saliva, net intestinal absorption of P, and excretion of endogenous P in feces. Three periods of 14 d each were used for three dietary treatments. Sheep were fed a pelleted hay diet (2 g of P/d; period 1) and supplemented with 2 g of P/d (period 2) or 4 g of P/d (period 3) via continuous intraruminal infusion of Na_2HPO_4. During period 1, .06 g of P/d were excreted in urine (3%) and 1.91 g of P/d were excreted in feces. As intake of P increased to 4 and 6 g/d, daily P excreted in urine remained low (3 and 7%). The major excretory pathway for P was feces. Increased intake of P resulted in increased net intestinal absorption of P (1.62, 2.64, and 4.26 g of P/d). The increased absorption occurred at a decreased efficiency (84, 66, and 71% of daily intake). Concentration of P in

plasma was greater in period 3 than in periods 1 and 2. Secretion of P in saliva was increased by the intraruminal infusion of P (7.62, 9.55, and 9.84 g of P/d).

Few experiments with cattle have been conducted to evaluate the effect of adequate or excess intake of P on excretion of P. Lamba et al. (1969) analyzed data from digestion studies carried out using 127 adult nonpregnant dry cows (41 diets) and 35 adult nonpregnant lactating cows (14 diets). Diets varied in type of forage and quantity and type of concentrate fed. Dry cows received a diet with less than 35 g of P/d (34 diets; 18.8 g/d) or more than 35 g of P/d (7 diets, 43 g of P/d). Intake of P varied from 7.9 to 54.1 g/d in dry cows and from 39.8 to 75.4 g/d in lactating cows (average 55 g/d). Daily excretion of P in urine ranged from .2 to 21 g/d in dry cows (mean 3.5 g/d) and from .3 to 30.5 g/d in lactating cows (mean 6.6 g/d). Excretion of P in feces ranged from 3.1 to 66.1 g/d in dry cows (mean 19.9 g/d) and from 2.6 to 40.9 g/d in lactating cows (mean 21 g/d). Correlations among fecal and urinary losses of P, P in milk, digestible P, and P balance of 55 diets in dry and lactating cattle were presented. The authors concluded that excretory pathway was not influenced by total quantity of intake of P and that balance of P was not related to intake of P. Inability to detect a relationship between intake and excretion of P in lactating cows may have resulted from low quantities of intake and large secretion of endogenous P. However, without knowing details of their statistical analyses it is difficult to fully interpret these data.

A more comprehensive evaluation for lactating cattle was reported by Hibbs and Conrad (1983). Fourteen experiments were summarized. Intake of P ranged from 20 to 60 g/d. Most experiments were with

smaller lactating cattle (350 to 450 kg). Intake of feed ranged from 9 to 14 kg/d and production of milk ranged from 4.5 to 17.8 kg/d. Phosphorus was excreted primarily via feces with less than 10% excreted in urine. When P content of milk was accounted for, less than 2% of total P excreted was in the urine.

Kincaid et al. (1981) assigned forty Holstein cows to one of four dietary concentrate treatments. Cows were fed assigned diets from parturition until 10 mo-postpartum. Ratio of concentrate to forage (grass-alfalfa silage) was 1:1. Concentrations of dietary P were .3 (low P) or .8% (high P) in concentrate portion. Estimated concentrations of P on a total mixed ration basis were 90 and 250% of recommended values (NRC, 1978). Concentrations of Ca were 1.0 or 1.7% of diet DM in concentrate. Estimated concentrations of Ca on a total mixed ration basis were 190 and 350% of the minimum recommended values (NRC, 1978). Concentration of dietary Ca did not affect yield of milk or 3.5% fat-corrected milk (3.5% FCM), or efficiency of feed conversion. Cows consuming reduced quantities of P produced 7% less milk. Cows on low P diets probably were consuming less than the recommended quantities of P during the first few week postcalving due to reduced feed intake and because of reduced concentration of P in concentrate. At 6 wk postpartum cows consuming the low P diet had lower concentrations of P in plasma (5.4 mg %) than cows consuming the high P diet (6.4 mg %). Based on concentration of P in plasma none of these cows were deficient in P.

Hormonal Factors and Phosphorus

The role of the endocrine system on absorption of P has received attention. Hormonal regulation of P occurs indirectly, as a result of regulation of Ca. Two hormones influence concentration of P in plasma. Parathyroid hormone (PTH) is secreted by the parathyroid glands when concentration of ionized Ca in the peripheral circulation is low. The second hormone involved is 1,25-dihydroxyvitamin D (1,25-$(OH)_2D_3$). In animals, vitamin D formation occurs when ultraviolet light irradiates skin and converts 7-dehydrocholesterol to vitamin D_3. In plants, light catalyzes conversion of ergosterol to vitamin D_2. Liver converts vitamin D_3 to 25-$(OH)D_3$, which is the major circulating form. Numerous other hydroxylations can occur with some physiological purpose. The enzyme 1-α-hydroxylase in the kidney is responsible for hydroxylation of 25-$(OH)D_3$ at carbon 1 to (1,25-$(OH)_2D_3$) which mediates Ca and P homeostasis.

In ruminants the most potent effecter of 1-α hydroxylase is PTH. Low concentration of plasma Ca stimulates production of PTH which then stimulates increased activity of the 1-α hydroxylase and increased synthesis of 1,25-$(OH)_2D_3$. Principle actions of PTH are on bone and kidney to increase concentration of plasma Ca and decrease concentration of plasma P_i. In nonruminants, increased concentration of PTH results in increased excretion of P in urine; in ruminants the kidneys play little role in excretion of P and salivary glands are more responsive to PTH for regulation and excretion of P (Mayer et al., 1968). Although excretion of P via urine is minimal in ruminants, it is sensitive to administration of PTH (Barlet and Care, 1972, Mayer et al., 1966, Mayer et al., 1968). Mayer et al. (1966) measured effect of administration of

PTH in intact and parathyroidectomized cows. Both types of cows responded to administration of PTH with decreased excretion of P in feces and increased excretion of P in urine. During administration of PTH, homeostasis of P was maintained by reduction in excretion of P in feces rather than by resorption of P from bone.

Wright et al. (1982) infused PTH directly into the arterial blood supply of a vascularly isolated parotid gland of anaesthetized sheep. Concentration of P in parotid saliva of infused sheep increased. Elevated concentration of P in saliva was associated with comparable decrease in concentration of bicarbonate. Phosphate and bicarbonate function as buffers in saliva entering the reticulorumen. Increased secretion of one of these buffers is accompanied by decreased secretion of the other to maintain pH in the reticulorumen.

Braithwaite (1980, 1981) evaluated effect of dose rate of 1-α hydroxy-cholecalciferol (1(OH)D_3) on metabolism of Ca and P in sheep. In the first experiment eight mature sheep received each of five treatments. Injection of 1(OH)D_3 intramuscularly (i.m.) provided four doses: 0, .02, .05, and .1 micrograms/d. A fifth treatment combined the high dose of 1(OH)D_3 and increased intakes of P and Ca (145 and 198 mg/d). This treatment was designed to determine if intake of Ca influenced absorption and retention of Ca or P. Data were expressed per kg of body weight. Intake of P and Ca were 100 and 110 mg/d (Braithwaite, 1980). Injection of 1(OH)D_3 increased rate of absorption and retention of P and Ca. The maximum response occurred at the lowest dose (.02 micrograms/d). Larger doses increased duration of response, but not the magnitude of response. Quantity of Ca consumed did not alter absorption or retention of Ca or P. Increased absorption and

retention of P was approximately the same for all doses of $1(OH)D_3$. Absorption of Ca increased with increasing doses of $1(OH)D_3$ (238, 490, 508, and 669 micrograms/d). Yet, increased absorption of P was approximately the same at all dose of $1(OH)D_3$. The author concluded that absorption of P was stimulated directly by $1(OH)D_3$.

In a second experiment $1(OH)D_3$ was or was not injected i.m. to sheep fed low or high quantities of Ca (37.5 or 197.5 mg of Ca/d) (Braithwaite, 1981). Amount of P in diets was 146.9 mg/d. Injection of $1(OH)D_3$ caused increased absorption of P from 55.2 and 59.8 to 69.7 and 78.0 mg/d for diets low and high in Ca. Retention of P was positive in injected sheep and increased from 4 to 20 mg/d when concentration of Ca increased from 37.5 to 197.5 mg/d. Increased retention of P in injected sheep given high Ca diet mainly was achieved by an increased rate of accretion of P into the nonexchangeable pools of bone and soft tissues. The author concluded that absorption of P was stimulated directly by $1(OH)D_3$ and not by intake of Ca. Retention of P was related to quantity of Ca absorbed

CHAPTER 3
EFFECTS OF CONCENTRATION OF PHOSPHORUS IN DIET ON CONCENTRATION OF PHOSPHORUS IN FECES OF LACTATING DAIRY COWS - A FIELD STUDY

Introduction

Environmental Perspective

Phosphorus has been identified as a troublesome nutrient which can adversely affects the ecosystem of Lake Okeechobee. Dairies located North of Lake Okeechobee have been identified as a major source of P entering the lake. Waters originating from the northern end of the lake contain a high concentration of P and contribute as much as 30% of total P entering the lake (Graetz, 1989). The Department of Environmental Regulation (DER) proposed implementation of Best Management Practices (BMPs) in 1981. The purpose of these BMPs was to reduce quantity of P entering Lake Okeechobee through its northern most inlet. These practices became law in 1987 and are known as the 'Dairy Rule' (Harvey, 1989). Waterways will be monitored and dairy managers will be required to contain P on their property.

Dairy Manager Perspective

A variety of alternate sources of feed (by-products) can provide adequate sources of energy and protein to meet nutrient requirements of lactating dairy cows. However, fine tuning of macro and micro minerals contained in such diets may be required. Coppock et al. (1972, 1976) reported that lactating dairy cattle were unable to regulate dietary

intake of P and Ca when fed diets low in P or Ca. Inability of cattle to regulate intake of these macro minerals was in contrast to reports that indicated animals were able to increase intake of P and Ca by consuming less traditional feedstuffs (Becker et al., 1933, Eckles et al., 1935). These seemingly conflicting reports are explainable. In those situations where animals increased intake of P or Ca, prolonged deficiency of these nutrients existed. Although the intake of deficient nutrients increased, actual regulation of intake of P and Ca to meet required needs has not been reported.

In diets formulated for dairy cattle, recommended concentrations of minerals have increased over time (NRC, 1978, NRC, 1989). Noteworthy in the most recent recommendations for dairy cows were recommendations specifically directed to the first 3-wk-postpartum. During this period, recommended concentrations of P, Ca, NDF and protein are .49, .77, 35, and 19% of diet DM (NRC, 1989). After the third week of lactation recommendations for these nutrients decrease to .41, .66, 28, and 18% of diet DM. Higher concentrations of P, Ca, NDF, and protein in diets were necessitated by the reduced intake of feed during the first 3 wk of lactation. Requirements of animals can be met more closely by increasing recommended concentrations of selected nutrients immediately postcalving.

One possible method to reduce flow of P from dairy farmland to surrounding environment is to reduce amount of P consumed by animals. This likely would require reducing concentrations of P in diets. However, before this is discussed it is important to develop a general understanding of how diets are formulated and why specific minerals, P in particular, are included in diets of lactating cows at higher levels

than seem necessary. Diets for dairy cattle often are formulated for least cost and minimum concentrations of nutrients. Energy and protein are costly components of diets and often are formulated at or near minimum accepted values. In some instances maximum concentrations of specific nutrients are utilized in diet formulation. Such nutrients include urea, fiber, and microminerals. Concentrations of minerals and vitamins are adjusted by addition of inorganic commercially prepared products. In South Florida, four macrominerals (potassium (K), magnesium (Mg), Ca, and P) commonly are fed above recommended concentrations (NRC, 1988). Research findings from Florida (Mallonee et al., 1985) indicated greater need for K during months when heat stress is present. Most recent are recommendations to increase dietary concentration of Mg when inorganic sources are used (O'Conner, 1987). Processing of magnasite ore, the source of inorganic Mg, alters biological availability of Mg (Beede, 1989). Therefore, increasing the amount fed of some supplemental sources of Mg is recommended due to decreased availability.

Formulating diets from by-product feeds can provide adequate energy, protein, and fiber, but, contents of minerals generally need to be altered. Two guidelines used in nonruminant nutrition have played key roles in diet formulation for dairy cows in Florida. Use of these guidelines has resulted in concentrations of dietary P and Ca that exceed those recommended by NRC. The first pertains to Ca:P ratio. In nonruminants recommended dietary Ca:P ratios range from 1.2:1 to 2:1. It is not necessary to maintain such a narrow Ca:P ratio for dairy cows. Recycling of P in saliva alters formulated dietary Ca:P ratio. As long as P is adequate, Ca:P ratios greater than 2:1 but less than 8:1 have

not been harmful to growth or production in ruminants (Dowe et al., 1957; Lamb et al., 1934, Ricketts et al , 1970). Ratios less than 1:1 have elicited reduced growth in steers (Wise et al., 1993). The second guideline from nonruminant nutrition which is not relevant to ruminant nutrition is availability of P bound to phytate. Discounting of P in cereal grains occurs when formulating diets for swine and poultry. Nonruminant nutritionists assume that the phytate bound P is unavailable for biological processes. Availability of P bound to phytate for ruminants will be addressed elsewhere (Chapter 9). When P in some feedstuff ingredients is discounted in formulating a diet for ruminants, additional P must be added as inorganic P to meet recommended concentrations established by NRC (1989).

An additional reason for elevated concentration of P in diets formulated in Okeechobee County must be considered, that of intake of DM. Recommended concentrations of dietary P are based on requirements of actual g of P needed/d. Quantity of P consumed equals concentration of P in diet multiplied by quantity of diet eaten. To illustrate this point, consider a 550 kg mature, nonpregnant, lactating cow producing 25 kg of 4% fat-corrected milk (4% FCM)/d. Requirement of P is 95 g/d [(.0143*550 + 99*25)/.5] (NRC, 1989). Recommended dietary concentration of P is .38% of diet DM. If this cow consumed DM at 3.6% of her body weight each day she would eat 20 kg of DM, providing 79 g of P/d. Intake of DM at 15, 20 or 25 kg/d would provide intakes of P of 57, 79, or 95 g/d if concentration of P was 0.38% of DM. In this example when feed intake was limited (i.e., less than 3.1% of body weight) dietary concentration of P must be increased to provide the cow with the 65 g of P/d that is required.

Most experiments dealing with concentration of P in diets of dairy cows were designed to determine the lowest acceptable concentration of P in diets or to determine adequacy of various Ca:P ratios. Although feeding experiments have evaluated effects of dietary concentration of P on milk production or growth, the data regarding excretion of P are limited. Holechek et al. (1985) evaluated the relationship between dietary and fecal P in an attempt to determine P status of beef steers on pasture. They evaluated six different forages and reported that concentration of P in feedstuff was associated highly with concentration of P in feces. Few experiments provided data from which total daily excretion (g of P/d) could be obtained (Hibbs and Conrad, 1983). Data in agricultural engineering literature have been used to define characteristics of dairy manure (Barth, 1985; Safley et al., 1984, 1985). Concentrations of nutrients in feedstuffs consumed by cows were not reported. Results were reports of random samples of feces or urine which were obtained from various dairies and were confounded with management and feeding characteristics. Data from these reports often have been used to estimate excretion of nutrients from dairy cows.

The objectives of this experiment were to determine if the concentration of P in feed affected concentration of P in feces and to estimate excretion of P of cows on commercial dairies.

Materials and Methods

Cooperating Farms and Feedmills

United Feed Cooperative (UFC) and Dairy Feeds, Inc. (DFI), both of Okeechobee County, FL, supplied feed for five dairies used in a field trial. The trial began November, 1986 and continued through September, 1987. Dry Lake Dairies (DLD) 1 and 2 were owned by Emmet Rucks and Sons

and McArthur Farms (MF) barns 1, 3, and 4 were owned by McArthur Farms, Inc. Dry Lake dairies received feed from UFC while MF dairies received feed from OFI. Each dairy milked between 700 and 1,300 cows per month; DLD-1, 1,000 cows, DLD-2, 750 cows; MF-1 and MF-4, 1,100 cows; and MF-3, 1,300 cows. Production and reproduction data were available for most cows sampled on MF. Dry Lake Dairy 2 began operation in October, 1996. Initially, some cows from DLD-1 were housed at the new facility. Springing heifers were purchased and as they calved, older cows were returned to DLD-1. Dry Lake Dairy-2 then was comprised only of first lactation cows, whereas other farms had primiparous and multiparous cows. Herd summaries for MF dairies were available weekly from the farm office. These summary sheets provided mean values for intake of silage and concentrate and production of milk.

Assigned Diets

Feed ingredient samples were obtained from cooperating feedmills in November (1996), February, May, and August (1997). These samples were used to calculate concentration of total P in concentrate mix. Concentrate portions of diets were formulated by feedmills to contain .42% P (DLD-2, MF-1) and .52% P (DLD-1, MF-3 and MF-4). Concentration of P in individual feed ingredients was determined by analyses of ingredients obtained every 3 mo. Concentration of P in concentrate portion of diets was calculated based on diet formulation obtained from feedmills and from total P content of ingredients as determined in the laboratory (Table 3-1). Mineral mix with minimum of 8.0% P was available free choice for cows at MF-3. Mineral mix was available at 57 to 85 g per head per day according to manufacturer's recommendation. Cows on other dairies did not have access free choice to mineral mix.

TABLE 3-1 CALCULATED CONCENTRATION OF PHOSPHORUS (% DM) IN CONCENTRATE PORTION OF DIETS FED AT FIVE DAIRIES DURING AN 11 MONTH PERIOD.[a]

Dairy[b]	Month										
	Nov	Dec	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept
DLD-1	.57	.57	.57	.57	.57	.57	.57	.57	.57	.57	.57
DLD-2	.47	.47	.47	.47	.47	.47	.47	.47	.47	.47	.47
MF-1	.75	.75	.45	.45	.45	.51	.51	.51	.51	.51	.51
MF 3	.72	.72	.71	.71	.71	.71	.71	.71	.71	.71	.71
MF-4	.72	.71	.71	.71	.71	.71	.71	.71	.71	.71	.71

[a] Values calculated from ingredient componnnt concentrations.

[b] DLD = Dry Lake Dairies, barns 1 and 2.
MF = McArthur Farms, barns 1, 3, and 4

containing P. Fnnd ingredients used to formulatn diets for DLD-1 and DLD-2 included cottonsend hulls, malt sprout pellets, hominy, whole cottonseed, wheat middlings, soybean hulls, corn meal, and cottonseed meal. From analyses of individual ingredients the low P diet actually contained .47% P on a DM basis. Thn high P diet fed at DLD-1 (57% total P) was achieved by addition of biofos (18% Ca and 21% P) to thn low P diet. Concnntrate mixes for MF used cottonseed hulls, malt sprout pellets, hominy feed, whole cottonseed, wheat middlings, soybean hulls, corn meal, and rice bran. Rice bran and multiphos (18% P and 34% Ca) were included in concentrate mixes high in P. Dietary concnntration of P for MF-1 was formulated for .42% of dint DM. Mnch of the P bound to phytate was not considnred available in rice bran and wheat middlings. Therefore, total P actnally was .72% of concnntratn DM. Content of P in concnntrate for MF-1 was based on total P, once the factor used to discount phytatn bound P was removed. Samples of corn and sorghum silage and sampins of fresh greenchop contained from 17 to 22% P (DM basis).

Sample Collection

Each month (November, 1986 through March, 1987, July and August, 1987) feed, fecal, and serum were obtained from up to 20 cows/dairy, selected at random. Samples of concentrate, silage or greenchop, and hay were obtained from feed troughs, silage wagons, and hay bales. Samples were stored in zip-lock bags. Fecal samples were retrieved per rectum. Fresh feces were stored in plastic containers, sealed, and kept away from direct sunlight. Samples of blood were taken from the tail vein of 10 to 15 cows/dairy, selected at random. Blood was collected into nonheparinized tubes. Tubes were placed on ice and blood was allowed to clot for 1 h. Tubes then were centrifuged at low speed in a portable tabletop centrifuge. Separated serum was pipetted into labelled 15 ml opaque polypropylene tubes and stored on ice. Hemolyzed samples were discarded. Concentration of P in serum was used as an indicator of P status of cows.

Sample Preparation

Feed and Feces. Feed and fecal samples were dried for at least 36 h in a 60 C oven. They were mixed every 12 h to prevent molding. They then were ground through a 1 mm stainless steel screen in a Whiley mill (Model 4), identified, and stored in whirl-pak bags. Preparation of stored samples for determination of P was according to the Method of the Association of Official Analytical Chemists for preparation of feedstuffs (AOAC, 1984). Duplicate samples of approximately 2 g were oven dried in 30 ml pyrex beakers for 36 h at 100 C, then dry-ashed for 4 h at 550 C. Beakers were allowed to cool prior to addition of 15 ml of 3N HCl, covered with watch glasses, and boiled for 10 min. After cooling, samples were filtered through Whatman 41 ashless filter paper

into 100 ml volumetric flasks and diluted to volume with deionized water (DI). After mixing thoroughly, subsequent dilutions (generally 2 ml into 25 ml volumetric) were made with 1N HCl. This represented a 1:625 dilution/g of dried feed or feces.

Serum and Milk. Serum and milk samples were prepared according to the Methods of Mineral Analysis for plant and animal tissues (Fick et al., 1979). One ml of serum or milk was deproteinized with 9 ml of 10% (w/v) trichloracetic acid (TCA). Samples were placed in an ice bath for 20 min then centrifuged at 2,500 rpm (770 x G) for 15 min at 13 C. Supernatant was decanted and stored in 15 ml opaque polypropylene tubes.

Determination of Phosphorus

Concentration of P was determined colorimetrically (Harris and Popat, 1954) using a spectrophotometer (Beckman DU-20 spectrophotometer, 660 µm). This technique depended on the conversion of P to phosphomolybdic acid and its subsequent reduction to produce a blue colored solution. The blue color resulted from colloidal reduced oxides of molybdenum.

Determination of Lignin

Acid detergent lignin (ADL) was determined for feed ingredients and feces according to Goering and Van Soest, 1970. Concentration of ADL in concentrate portion of diets was calculated based on formulated quantities of feed ingredients. Estimated excretion of F/d was calculated using ADL as an indigestible marker (Kane et al., 1953) and the estimated intake of DM as determined from farm records.

Figure 3-1. Average daily intake of feed dry matter (DM) of cows plotted on a weekly basis from November, 1986 through December, 1987 for three McArthur dairy farms (MF).

Average intake of DM (kg/d)
23
22
21
20
19
18
17
16
15
14
13
MF-1
MF-3
MF-4
Nov Dec Jan Feb Mar Apr May Jun July Aug Sep Oct Nov Dec

Results and Discussion

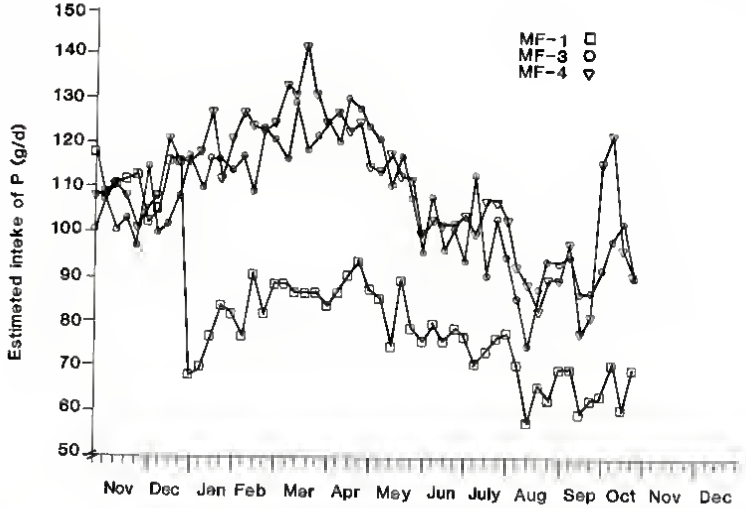
Concentration of Phosphorus in Feces

Average daily intakes of concentrate for 7 d periods were available for MF dairies. Intake of DM by cows ranged from 13 to 22 kg/d (Figure 3-1). Decreased intake of DM occurred during warm, humid days. Intake of DM for concentrate and roughage was in an 80:20 ratio. Estimated average daily intake of P was the summation of P from concentrate and roughage, assuming negligible contribution of P from grazing. Average intake of P by cows fed diets containing high concentration of P was > 100 g/d from November through June and between 80 and 100 g/d from July through September (Figure 3-2). Estimated intake of P by cows at MF-I, fed diets low in concentration of P, generally was between 70 and 90 g/d once concentration of P was adjusted from the higher concentration to the lower concentration of P, as described previously (Assigned Diets, p 57). Suggested requirement for P was 65 to 85 g/d for cows producing 25 to 35 kg of 4% FCM/d (NRC, 1989). Hence, on the average, cows consumed greater than adequate quantities of P.

Fecal sample data were evaluated by two methods. In the first case a subsample of data was evaluated (276 or 277 observations; parity number could not be identified for one observation). Data in the subset had corresponding information regarding desired parameters. Parity, age at calving, days in milk, and milk production were known for these data observations. It was hoped that milk production and day of lactation were indicative of intake of DM. Data not included in this subset generally lacked information on milk production or parity.

The first series of analyses included concentration of P in feces and day of lactation (linear and quadratic), milk production, age,

Figure 3.2 Average daily intake of Phesphorus (P) of cows plotted on a weekly basis from November, 1986 through December, 1987 for three McArthur dairy farms (MF). Average intake based on dairy records of consumption of concentrate and silage.


MF-1
MF-3
MF-4
Estimated intake of P (g/d)
150
140
130
120
110
100
90
80
70
60
50
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
July
Aug
Sep
Oct
Nov
Dec
65

dairy, month, parity, and breed and interaction terms dairy*month, dairy*breed, month*breed and dairy*month*breed and accounted for 60% of variation (R^2=.60) (Appendix A, Table A-1). Dairy, month, and parity were considered discrete and the remaining factors were classified as continuous. Analyses were iterated after deletion of individual nonsignificant main effects and corresponding interaction terms. The reduced models thus were analyzed to identify additional nonsignificant terms.

Breed, age, parity, and milk production of cows accounted for about 1% of observed variation in concentration of P in feces (R^2 decreased from .60 to .59). Although day of lactation (quadratic) was significant, the linear and quadratic terms were removed to determine the degree to which they explained observed variation. Values for R^2 decreased from .59 to .54. Least squares mean concentrations of P in feces for all dairies in all months were not computed for the reduced data subset because of missing data.

The reduced model, which included dairy, month, and the interaction term, was utilized with all data observations included (n=688). The R^2 was .53. This model was used to determine least squares mean concentrations of P in feces for months and for all five dairies (Table 3-2). Dairy, month, and dairy*month were significant ($p < .01$). If dairies were ranked by average concentration of P in feces, the ranking would change from month to month. This resulted, in part, because of elevated concentrations of P in concentrate fed at MF-1 during November and December. Concentration of P in concentrate fed at MF-1 was reduced from .75 to .45% during January and then increased to .51% in April. Orthogonal comparisons of concentration of P in feces by dairies

detected differences in concentration of P in feces of cows at DLD and cows at MF (Table 3-3). This probably reflects differences in amount of feed cows consumed daily and differences in concentration of P in concentrate portion of diet as diet and farm management were confounded. Concentration of P in concentrate fed at DLD was less than .57%. Also, cows at DLD consumed an unknown quantity of fresh greenchop daily, whereas, cows at MF consumed 2 to 3.5 kg of silage DM daily. Concentration of P in concentrate fed at MF-3 and MF-4 was greater than .70%.

TABLE 3-2. LEAST SQUARES MEAN CONCENTRATION OF P IN DRIED FECES (MG/KG, PARTS PER MILLION)[a] FROM COWS AT FIVE DAIRIES DURING SEVEN MONTHS

	Month						
Dairy[b]	Nov	Dec	Jan	Feb	Mar	July	Aug
DLD-1	7105	5625	5558	6204	6959	9710	9662
DLD-2	5592	6882	6047	5691	5859	6144	8192
MF-1	11347	9158	5419	5513	5021	9469	7072
MF-3	9267	8677	7772	10100	9428	10857	10366
MF-4	8781	9154	6976	8925	8215	11039	9275

[a] Least squares anelysis of variance located Appendix A, Table A-7 (n=688)

[b] DLD = Dry Lake Dairies, barns 1 and 2
MF = McArthur Farms, barns 1, 3, and 4

SE ranged from 403 to 451 mg of P/kg of DM.

TABLE 3-3. ORTHOGONAL COMPARISONS[a] OF CONCENTRATION OF P IN FECES BY DAIRY ADJUSTED FOR MONTH.

Contrast	DF	Type III SS	F	PR
DLD vs MF[b]	1	560049749.08	172.39	.0001
DLD-1 vs DLD-2	1	66911762.39	20.60	.0001
MF-1 vs MF-3 & 4	1	247911789.39	76.31	.0001
MF-3 vs MF-4	1	22393992.63	6.89	.0089

[a] Least squares analysis of variance located Appendix A, Table A-7 (n=688)

[b] DLD = Dry Lake Dairies, barns 1 and 2.
MF = McArthur Farms, barns 1, 3, and 4

Other orthogonal comparisons of concentration of P in feces indicated differences between DLD-1 and DLD-2 (higher vs lower P), MF-1 and MF-3 or MF-4 (lower vs higher P), and MF-3 and MF-4 (higher P plus free choice mineral vs higher P without free choice mineral). Adjusted for month, least squares mean concentrations of P in feces (g/kg of DM) by dairy were 7318 (DLD-1), 6344 (DLD-2), 7571 (MF-1), 9495 (MF-3), and 8910 (MF-4). Standard errors ranged from 152 to 159 ppm.

Least squares mean concentrations of P in feces by month adjusted for dairy were greatest for July (9443) and August (8913 mg of P/kg of DM). Mean concentrations for November through March were 8418, 7899, 7435, 7287, and 7097, SE ranged from 180 to 185. Concentrations during November and December were elevated for MF 1 because the concentration of P in concentrate portion of the diet had not yet been reduced to the desired concentration. Concentration of P in feces collected during July and August appeared greater than that in feces collected during cooler months. Feed intake of cows was reduced during August, September, and October. It is possible that greater digestibility of DM occurred due to reduced total intake of feed. This would result in the excretion of less manure DM, but manure DM which contained greater concentration of P. Concentration of P in saliva tended to decrease when production of saliva increased (Bailey and Balch, 1961a, 1961b). If production of saliva was increased during hot weather, excretion of endogenous P would be greater during months of hot weather compared to production of saliva during other months.

In a second set of analyses concentration of P in concentrate portion of diets replaced dairy and month in the model (Appendix 8, Tables 8-1 through 8-7). Concentration of P in diet was considered a

continuous independent variable to evaluate associations between concentration of P in DM consumed and concentration of P in DM excreted. Models were reduced, as in the previous set of analyses. For these analyses it was assumed that all cows had consumed equal amounts of DM of the same composition. Variation associated with DM consumption can be explained, in part, by milk production and day of lactation. The greatest R^2 obtained from the complete model was .46. A reduced model which considered only effects of day of lactation (linear and quadratic), milk production (linear), and concentration of P in concentrate portion of feed explained 45% of the observed variation in concentration of P in feces. Parameter estimates and respective SE were calculated (Table 3-4). These estimates were used to calculate the expected concentration of P in fecal DM. Based on these values the expected concentrations of P in feces from concentrates containing .50 and .60% P were 6093 and 7339 mg of P/kg of DM.

TABLE 3-4. ESTIMATES OF COEFFICIENTS* AND STANDARD ERRORS OF ESTIMATES FROM ANALYSIS OF CONCENTRATION OF PHOSPHORUS IN FECES.

Parameter	Coefficient	SE
Intercept	1487.00	782.33
Day of lactation (DIM)	-3.71	2.94
DIM*DIM	0.021	0.006
Milk	-64.55	16.2
PDiet	1246102.23	101079.40

* Least squares analysis of variance found in Appendix 8, Table B-5. Milk = yield (kg/d), PDiet = concentration of P in concentrate.

Model: $P = \mu + DIM + DIM*DIM + MILK + PDIET$.

A third set of analyses evaluated variation in concentration of P in feces from cows at MF (277 samples) with regard to average daily intake of P (Appendix C). Only data for MF were considered since no estimates of intake of concentrate were available for cows at DLO-I or

DLD-2. It was assumed that each cow on MF dairies consumed the same amount of concentrate (feed delivered to farms divided by number of cows in milk). Intake of P other than that from concentrate or silage was not quantified. This would include P consumed as pasture and P consumed in free choice mineral mix (MF-3). Intake of P from pasture probably did not contribute more than 5 g of P/d. Intake of P from mineral mix available free choice to cows at MF-3 did not exceed 7 g if consumed at 95 g of mineral mix/d. It was assumed that increased intake of P resulted from increased concentration of P in concentrate and not from increased quantity of concentrate consumed. This assumption was based on records obtained from MF dairies. It also was assumed that digestibility of DM was not altered as concentration of P in diet increased. Individual cow variation could not be measured since cows were group fed. Linear and quadratic effects of average daily intake of P and linear effects of days in milk and level of milk production were significant. Parameter estimates were derived (Table 3-5). Estimate of

TABLE 3-5 ESTIMATES OF COEFFICIENTS[a] AND STANDARD ERRORS OF ESTIMATES FROM ANALYSIS OF CONCENTRATION OF PHOSPHORUS IN FECES.

Parameter	Coefficient	SE
Intercept	- 30657.08	4258.85
Day of lactation (DIM)	1.19	3.06
DIM*DIM	0.019	0.007
Milk	53.28	17.71
Pin	704.68	76.94
Pin*Pin	- 3.04	0.36

[a] Least squares analysis of variance found in Appendix C, Table C-5. Milk = yield (kg/d), Pin = quantity of P consumed (g/d)

Model: $P = \mu + DIM + DIM*DIM + MILK + Pin + Pin*Pin$

concentration of P in feces for cows 60 d in milk, producing 22.7 kg of milk/d and consuming 75 g of P/d was 3877 mg of P/kg of DM. This value

wes less than that found in any of the fecal samples analyzed. Lowest concentretion of P in feces analyzed wes approximately 4200 mg of P/kg of DM. The majority of fecal samples were collected from cows consuming 80 to 120 g of P/d. As intake of P in DM consumed increased from 80 to 100 g of P/d, estimated concentration of P in feces increased from 5044 to 8194 mg of P/kg of DM. These estimates better approximated concentrations of P in samples collected and analyzed. As intake increased 25% from 80 to 100 g of P/d, estimated concentration of P in fecal DM increased 62% from 5044 to 8194 mg of P/kg of DM. Large SE associated with coefficients and low R^2 for the model (.42) may be responsible for estimates less than those observed from samples collected and analyzed. Cows sampled may not have been representative of the average cow within each herd. Cows sampled may have consumed more P than the estimated value based on farm records

Estimation of Excretion of Phosphorus (g/d)

The final series of analyses accounted for variation in estimated excretion of fecal P (g/d). To estimate excretion of P in feces, an indigestible marker was used to determine digestibility of feedstuffs (Kane et al., 1953; Reid et al, 1950). Lignin, which occurs naturally in feedstuffs, was used as the marker. The same assumptions as for feed intake and composition of feed consumed were applicable (all cows consumed an average amount of feed of equal composition). It was assumed that lignin was neither degraded in nor excreted into the GIT and that no pasture was consumed. Any lignin consumed and not accounted for would result in an underestimate of excretion of DM and therefore, a conservative estimate of excretion of P. If components of lignin were degraded in the GIT then the concentration of lignin in feces would be

loss than it should be and this would yield a greater indigestibility of feed and an overestimate of excretion of DM.

The ratio of lignin in the feedstuff to lignin in the feces permitted calculation of the percent of the diet which was not digested. This value multiplied by average intake of DM provided an estimate of DM excreted in feces. Quantity of DM excreted multiplied by concentration of P in the fecal samples obtained was used to estimate daily excretion of P.

Data from MF-1, MF-3, and MF-4 were used in these analyses since estimated intake of silage by cows was available. Month and dairy*month were significant ($p < .01$) sources of variation for estimated excretion of P. Least squares analysis of variance tables are in Appendix D. Only 35% of the variation in estimated excretion of P (g/d) was partitioned by dairy, month, and interaction term dairy*month. Estimates of least squares mean daily excretions of P by month for the three dairies were obtained (Table 3-6). The decrease in concentration of P (from .75 to .45%) in concentrate portion of diet at MF-1 in December and January was reflected by decreased estimates of excretion of P in feces (99.7 to 39.1 g/d).

TABLE 3-8. LEAST SQUARES MEAN ESTIMATES OF EXCRETION OF P IN FECES (G/D) BY DAIRY AND MONTH.

	Month			
Dairy[a]	Nov	Dec	Jan	Feb
MF-1	73.82[b]	99.85	39.09	45.57
MF-3	55.35	60.50	51.41	70.14
MF-4	62.91	51.74	46.29	59.23

[a] MF = McArthur Farms, number denotes barn.

[b] Least squares analysis of variance table located in Appendix D, Table D-1.
SE ranged from 3.37 to 3.90 g/d.

Determination of Phosphorus Status of Cows

Concentration of P in serum was used to indicate status of P in cows. Forty-four percent of variation in concentration of P in serum was attributed to dairy, month, and the interaction (Table 3-7). Overall least squares mean concentration of P in serum was 6.1 mg %.

TABLE 3-7. LEAST SQUARES ANALYSIS OF VARIANCE FOR CONCENTRATION OF P IN SERUM (Mg %).

Source	DF	Type III SS	F	PR
Month	6	277.64	32.84	.0001
Dairy	4	17.60	3.12	.0148
Month*dairy	24	302.34	8.94	.0001
Error	548	772.26		

Mean concentration of P in serum did not differ between cows of the two ownerships DLD or MF ($p = .35$). Adjusted for month, cows at DLD-2 (lower P) had greater concentrations of P in serum than cows at DLD-1 (higher P) (6.33 vs 5.98 mg %; $p = .02$) and cows at MF-1 (lower P) had lower concentrations of P in serum than cows at MF-3 or MF-4 (5.89 vs 6.28 and 6.01 mg %; $p = .07$). Standard error for least squares mean concentration of P in serum by dairy was .11 mg %. Least squares mean concentrations of P in serum for dairy month categories ranged from 4.04 to 7.86 mg % (Table 3-8). All serum values were within acceptable range for lactating dairy cows (Blood and Henderson, 1968).

TABLE 3-9. LEAST SQUARES MEAN CONCENTRATIONS OF P IN SERUM (Mg %) BY DAIRY AND MONTH.

			Month				
Dairy[a]	Nov(a)[b]	Nov(b)	Dec	Jan	Feb	Mar	July
DLD-1	5.69	4.04	6.05	7.59	5.94	5.34	5.95
DLD-2	5.15	5.24	7.15	7.42	7.07	7.24	5.00
MF-1	6.88	6.41	5.42	5.04	5.17	6.72	5.57
MF-3	6.91	5.21	5.80	7.94	5.42	6.99	5.72
MF-4	6.55	4.29	5.10	6.39	6.61	7.86	5.22

[a] DLD = Dry Lake Dairies, barns 1 and 2
MF = McArthur Farms, barns 1, 3, and 4

[b] Nov(a) November 10, 1986; Nov(b) November 29, 1986
SE ranged from .27 to .38 mg %

Summary

Concentrations of P in concentrate portion of diets fed to lactating dairy cows on commercial dairies were altered to determine affects of dietary concentration of P on concentration of P excreted in feces. Average intake of P by cows at MF-3 and MF-4 was generally between 80 and 120 g of P/d. Average intake of P by cows at MF-1 that consumed concentrates lower in P was between 50 and 80 g of P/d. Average intake of P for cows owned by MF was in excess of the required intake of 55.7 g of P/d (suggested by the NRC) for a mature 550 kg, nonpregnant cow, producing 20 kg of 4% FCM. Concentration of P in feces was decreased when concentration of P in concentrate was decreased. The magnitude of the decrease depended on ownership. Adjusted for month, cows fed diets lower in concentration of P at DLD-2 or MF-1 had lower concentrations of P in feces than cows fed concentrates with higher concentrations of P (DLD-1, MF-3 or MF-4). Cows allowed access to free choice mineral containing a minimum of 9.0% P (MF-3) had greater concentrations of P in feces than cows fed a similar diet and not allowed access to free choice mineral (MF-4). The major dietary

difference between cows at MF-3 and MF-4 was availability of free choice mineral mix. Dairy, month, and the interaction term dairy by month were responsible for 53% of variation in concentration of P in feces. Concentration of P in diet (PDiet) or estimated intake of P (g/d) explained less of the variation in concentration of P in feces than dairy, month, and dairy by month interaction. When lignin was used as an indigestible marker to estimate excretion of DM, estimated excretion of P was 58.9 g/d from cows consuming between 80 and 120 g of P/d. Average concentration of P in serum was 6.1 mg % compared to acceptable values between 3 and 8 mg %.

CHAPTER 4
EFFECTS OF CONCENTRATION OF DIETARY PHOSPHORUS
ON ROUTE OF EXCRETION OF PHOSPHORUS

Introduction

When intake of a nutrient is altered is excretion of the nutrient altered proportionally? Efficiency of utilization of most nutrients decreases as intake increases. This is true for P. Intake of dietary P, age and growth status of the animal and previous level of P intake are major determinants of efficiency of utilization of P. Unlike many nutrients, intake of P is not regulated wholly by body needs. Dairy cows with mild deficiency of P do not regulate intake of P to body needs (Coppock et al., 1972; Coppock et al., 1976). Yet, animals suffering from chronic signs of P deficiency will exhibit pica to increase intake of P (Becker et al., 1933; Eckles et al., 1935).

Does the relationship between intake and excretion of P differ above and below maintenence? Estimated requirement of P for a mature, 550 kg, nonpregnant cow producing 20 kg of 4% FCM is 55.7 g of P/d and is calculated from the equation [(0.143*live weight + 99*FCM)/ 5] where .5 is the efficiency of absorption (NRC, 1989). Lomba et al. (1969) summarized 14 experiments in mature, lactating, nonpregnant dairy cows producing 11 to 20 kg of milk/d. They reported no relationship between dietary intake of P and excretion of P. It is important to note that only in four of these experiments was P fed at > 60 g of P/d. Consequently, few of the experiments reported by Lomba et al. (1969)

evaluated diets where amounts of P fed were comparable to what is required by dairy cows producing greater than 20 kg of 4.0% FCM.

The objectives of this experiment were to determine if excretion of P was effected by intake of P and to determine if inert materials in feeds can be used to estimate total excretion of feces and thus be an indirect measure of excretion of P. Use of an inert marker in feed would provide a tool for monitoring excretion of P by cows under field conditions.

Materials and Methods

Animals

Twelve multiparous lactating Holstein cows were used in a 13 wk continuous trial. Cows were more than 60 d in milk at the beginning of the experiment. During the first 4 wk (weeks -4 through -1), all cows consumed a diet containing .41% P on a DM basis. At the end of week -1, cows were assigned randomly to one of three dietary concentrations of P: .30, .41 or .56% of diet DM and were referred to as low (L), medium (M), or high (H). These concentrations represented 73, 103, and 147% of recommended concentration of P in diet DM for a 590 kg dairy cow producing between 21 and 32 kg of 4.0% FCM/d (NRC, 1989). The highest concentration of P (.56% of diet DM) best represented concentration of P in feed fed to dairy cows in Okeechobee basin dairies.

Formulation of Diets and Feeding of Animals

The basal diet of .30% P on a DM basis was formulated from analyses of ingredients of feedstuffs that were obtained from the New York Dairy Herd Improvement Forage Testing Laboratory. Diet consisted of corn, corn silage, cottonseed hulls, soybean meal, and minerals (Table 4-1). Chromium was added to the diet in the form of Cr_2O_3 as an indigestible

marker. The two greater dietary concentrations of P (.41 and .56%) were achieved by addition of dicalcium phosphate to diet L.

TABLE 4-1. INGREDIENT COMPOSITION OF BASAL DIET.

Ingredient	% of DM
Ground corn	45.12
Corn silage	20.19
Cottonseed hulls	17.30
Soybean meal (48%)	13.48
Urea	1.73
$CaCO_3$	1.02
Trace mineral salt	.84
Magnesium oxide	.32
Dicalcium phosphate	.00
Total	100.00

Concentrates were mixed twice weekly during weeks -4 through -1 and every 3 wk thereafter. Samples of concentrates were taken each time concentrates were mixed and analyzed for concentration of P. Corn silage was sampled three-times weekly for determination of DM and concentration of P. Quantity of corn silage fed was adjusted for content of DM. Cottonseed hulls were sampled biweekly for determination of DM and concentration of P. Dietary concentrations of nutrients were calculated based on results from analyses of individual ingredients from the New York Dairy Herd Improvement Forage Testing Laboratory. Concentration of P was determined based on mean values obtained from laboratory analyses (University of Florida) of samples of concentrate, corn silage, and cottonseed hulls (Table 4.2).

Concentrate, cottonseed hulls, and corn silage were mixed and fed as total mixed rations (TMR) twice daily at 0830 and 1600 h. Cows were trained to use individual electronic feeding gates (American Calan Inc.) during the 10 d prior to the beginning of the experiment (mid-March). At this time, cows were fed the M diet ad libitum. Cows were

TABLE 4-2. NUTRIENT COMPOSITION OF DIETS CALCULATED FROM SAMPLES ANALYZED AT NEW YORK DAIRY HERD IMPROVEMENT FORAGE TESTING LABORATORY.[a]

Nutrient[b]	DIETARY TREATMENT[c]		
	L	M	H
	percent of dry matter		
Crude Protein	16.1	15.4	15.4
ADF	23.1	22.6	21.2
NDF	35.8	36.0	36.44
TDN	75	75	77
NE_l	.70	.71	.72
Calcium	1.17	1.45	1.71
Potassium	1.03	1.01	1.04
Phosphorus[d]	.30	.41	.55
Magnesium	.41	.40	.37
Sodium	.49	.48	.50

[a]Ithaca, New York.

[b] ADF = Acid detergent fiber (%).
NDF = Neutral detergent fiber (%).
TDN = Total digestible nutrients (%).
NE_l = Net energy for lactation (Mcal/kg).

[c]Diets L, M, and H represent low, medium, and high concentrations of P.

[d]Concentration of P determined on samples of feed ingredients analyzed in Dairy Science nutrition laboratory.

housed in a freestall or shade barn with access to a sand exercise lot during 9 of the 13 wk. Cows were relocated to a flatbarn equipped for total collection of excreta during weeks -1, 3, 6, and 9 (Figure 4.1). Evaporative cooling system was used in the flatbarn during balances at weeks 6 and 9. This maintained an ambient temperature of less than 32 C. Fresh water was available ad libitum at all times. Intake of DM was limited to the appetite of the cow which consumed the least amount of DM. Therefore, intake of P would not be confounded with intake of DM. Cows consumed 23 kg of DM when housed in either the freestall or the shade barn. Feed intake was reduced to 20 kg of DM/d one day prior to relocation of cows to the flatbarn and intake was maintained at 20 kg/d while cows were housed in the flatbarn. Intake was reduced in

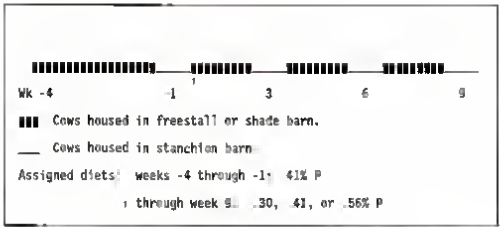


Figure 4-1. Experimental design and protocol.

anticipation of a decrease in intake expected due to relocation. Daily consumption of 20 kg of DM equated to 60, 82, and 112 g of P/d. When cows were housed in the flatbarn, water consumption was recorded from individual water cups at 1000 h daily.

One cow (diet M) suffered a displaced abomasum and was removed from the experiment during the first day of the third balance. One cow consuming the L diet provided excreta for 2, 3, and 4 d of the third balance then stopped eating. She was moved from the flatbarn to the shade barn. Intake of feed returned to normal by the end of the third balance and she continued on the experiment.

Sample Collection

Cows were weighed once daily, after the morning milking, on Friday, Saturday, and Sunday prior to each balance. After Sunday morning milking cows were haltered and relocated to stanchions in the flatbarn. Urinary catheters (Foley, 75cc) were inserted through the urethra into the bladder and the balloon was inflated with 80cc of water to retain

the catheter. Tygen tubing was attached to the open end of the catheter and extended into five-gallon plastic containers where urine accumulated.

Each balance consisted of 7 d of collection of all excreta. Samples from the first day were not used. Samples were composited in two periods: d 2, 3, and 4, and d 5, 6, and 7. Use of periods provided information on within cow variation. If variation within a cow-period were small, periods could be pooled within balance and provide replicate samples within cow-balance.

Milk. When cows were housed in the flatbarn they were milked in place at 0700 and 1800 h. Milk was collected and weighed in a stainless steel portable milking unit. Milk samples from individual cows from each milking were stored in whirl pak bags and frozen (-20 C). Samples of milk were composited at 1 g/ 45 kg of milk produced.

Feces. For 7 consecutive d, from Monday 0900 h through the following Monday at 0800 h, all excreta were collected, weighed, and sampled. Composite samples were taken daily for each of the 7 d. Manure was collected in individual pans located behind each cow. Pans and contents were weighed once daily at 0830 h, then contents were mixed and sampled for determination of DM. Dried samples were labelled and stored for analysis. Dried manure samples were ground through a 1 mm stainless steel sieve and composited at 5% of daily excretion of DM.

Urine. Urine was weighed at 2300 and 0530 h and composited by day at 1 cc of sample/ 45 kg of urine excreted and daily composites were frozen (-20 C).

Sample Analyses

Milk. Milk samples were thawed in warm water (25 C), emptied into an industrial Waring blender, and mixed at low speed. Duplicate samples of approximately 2.5 g of well-mixed milk were taken while blending and transferred to 50 ml tared pyrex beakers. Exact milk sample weights were recorded and beakers were placed in a 100 C oven overnight to evaporate water. Beakers then were placed in a muffle furnace and samples were ashed at 500 C for 4 h. Samples were solubilized and analyzed for P, as described previously (Chapter 3).

Urine. Urine samples were thawed at room temperature. Duplicate 30 g samples of urine were weighed into 50 ml tared pyrex beakers. Concentrated HCl (12.1N) was added dropwise until foaming ceased. Beakers were heated on a hot plate until 5 to 10 ml of liquid remained, then they were placed in a 100 C oven overnight. Short glass rods were used to mix dry contents in beakers prior to ashing at 500 C for 4 h. Samples were solubilized in 10 ml of 3N HCl, filtered into 10 ml volumetric flasks, brought to volume with distilled water, and concentration of P determined as described previously (Chapter 3). Eight urine samples from the first balance were lost and not available for determination of concentration of P.

Blood. Samples of whole blood were obtained from cows once weekly after am milking, but before am feeding. Samples were collected from the tail vein into nonheparinized tubes. Samples were stored on ice, allowed to clot for up to 1 h, then centrifuged at 2,500 rpm (770 x G) for 15 min, serum aliquoted into 15 ml opaque polypropylene tubes and stored on ice while being transported to laboratory. Samples with hemolysis were discarded and replacement samples were obtained the

following day. Samples of serum were precipitated with 10% TCA and analyzed for concentration of P, as described previously (Chapter 3).

In addition to determination of concentration of P, manure samples also were analyzed for concentration of Cr (Williams et al., 1962) and ADL (Goering and Van Soest, 1970). These markers were used to estimate quantity of DM excreted daily (Smith and Reid, 1955) by the equation:

$$\text{Fecal output (g DM/d)} = \frac{\text{marker consumed (g/d)}}{\text{concentration of marker in feces (g/g DM)}}$$

Estimated quantity of P excreted daily was calculated by multiplying concentration of P in feces by calculated output of feces. Calculated quantity of P excreted using Cr and ADL were compared to actual values of P excreted, as determined by total collection technique.

Actual apparent digestibility of DM (%) was determined using the relationship:

$$\%\ \text{digestibility of DM} = \frac{\text{DM intake (kg)} - \text{DM excretion (kg)}}{\text{DM intake (kg)}} * 100\%$$

This was compared to calculated coefficients of apparent digestibility using markers (Kane et al., 1953; Reid et al., 1950).

$$\text{apparent nutrient digestibility (\%)} = 100 - \frac{100 * a * x \text{ in feces}}{b * x \text{ in feed}},$$

where: a = % marker material in feed,
b = % marker material in feces;
x = % nutrient.

Statistical Analyses

Data were analyzed by method of least squares analysis of variance using the General Linear Models procedure (SAS, 1985). Variation in excretion of P through the various pathways was attributed to diet, cow(diet), and period or balance. Cow(diet) was the error term used to test for treatment significance.

Results and Discussion

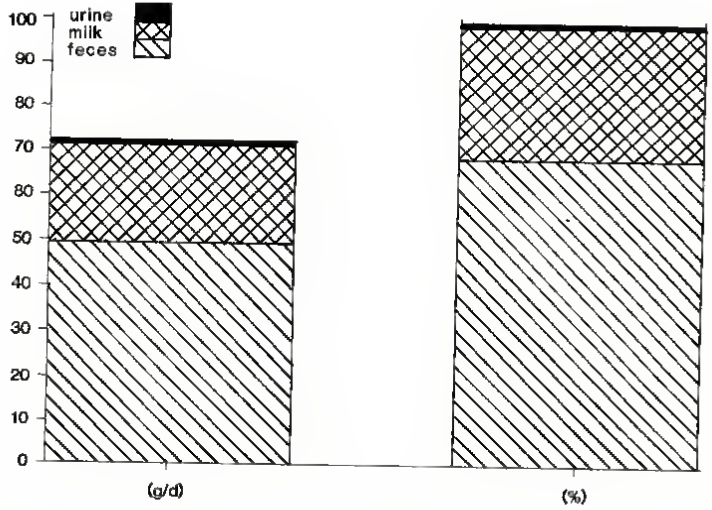
Total Collection Technique

Data obtained from the first balance (week -1) represented quantities and routes of excretion of P when all cows were fed the same diet (41% P, 82 g of P/d). These data were analyzed separately from the remaining three balances.

As indicated, two composite samples were made for each balance and each composite represented a period. Excretion of P in feces during the two periods of the first balance differed (Appendix E, Table E-1). This phenomenon may have resulted from a change in physical environment from the shade barn to the flatbarn. Content of P in fecal samples for periods within balances 2, 3, and 4 did not differ from each other. Quantity of P excreted in milk or urine for the other composites within a balance did not differ. There were no differences in total P excreted between periods within a balance. Results for the two periods within a balance were pooled and were replaced by balance number for analyses (Appendix F). The model for balance 1 data consisted of diet, cow(diet), and error. Cow(diet) was the error term used to test for significance of diet.

Cows consumed 82 g of P/d during weeks -4 through -1. As expected, the diet cows subsequently would be assigned to did not affect route of excretion of P during the first balance. Phosphorus was excreted in feces (49.6 g/d), milk (21.9 g/d), and urine (0.8 g/d; Figure 4-2). These values corresponded to 68.6, 30.3, and 1.0% of total daily excretion of P. Little P was excreted in urine. This agreed with work by Hibbs and Conrad (1983) who reported that less than 2% of total P excreted was in the urine, but > 70% of P was excreted in feces of

Figure 4-2 Least squares mean daily excretion of phosphorus (g/d and % of daily excretion) by dairy cows during one intake-excretion balance study. Balance was 7 d in duration. Twelve cows each consumed 82 g of P/d for three wk prior to and during balance.


urine
milk
feces
100
90
80
70
80
50
40
30
20
10
0
(g/d)
(%)

lactating cows. When cows retained > 10 g of P, the amount of P excreted in milk represented a larger percentage of total P excreted (30 to 40%).

Variation in excretion of P during balances 2, 3, and 4 was partitioned using the model diet, cow(diet), balance and the interaction of diet by balance, where cow(diet) was the error term used to test for significance of diet. Interaction between diet and balance for excretion of P in feces and urine was not significant ($p > .10$). Excretion of P in feces and urine differed due to diet and balance ($p < .01$). Variation in excretion of P in milk and production of milk was attributed to balance and the interaction of diet by balance. The differences due to balance were expected due to increased days in milk. Differences due to the interaction of diet by balance can be explained, in part, by reduced milk yield of cows consuming L during balance 2 and of cows consuming diet M during balance 1. Cows in these groups were treated for clinical mastitis during balances 2 and 1. There was a trend for less persistency of milk yield in balances 1, 2, 3, and 4 by cows consuming diet M (23.2, 24.4, 20.8, and 20.2 kg of milk/d) when compared to the persistency of milk yield by cows consuming diet H (24.1, 23.1, 21.0, and 21.3 kg of milk/d). Correction for content of fat was not made. Apparent large declines in milk yield from balance 2 to balance 3 was associated with increased ambient temperature and humidity during that time.

Cows that were assigned to .41% P diet (M) maintained a fairly constant excretion of P in feces and urine (Table 4.3). Excretion of P via feces increased 10.6% from balance 1 to balance 4 (Figure 4.3). Increased excretion of 4.8 g of P/d in feces was associated with

decreased milk production and a 19.3% (4.2 g of P/d) decrease in excretion of P in milk. Total excretion of P was less than intake (68.3 g of P excreted vs 82 g of P consumed) which indicated cows retained 13.7 g of P/d.

TABLE 4-3 LEAST SQUARES MEAN GRAMS OF PHOSPHORUS EXCRETED DAILY BY LACTATING HOLSTEIN DAIRY COWS DURING BALANCES (B) 1 THROUGH 4 BY DIET AND ROUTE OF EXCRETION.[a]

Route	B 1	B 2	B 3	B 4	change[b] (g/d)	change[b] (%)
Low P diet (.30% ; 60 g/d)						
Feces	53.74	32.39	33.57	41.57	-12.17	-22.65
Milk	19.68	18.44	17.96	17.79	1.89	- 9.60
Urine	.52	.87	.61	.63	.11	21.15
Total	71.84	51.70	52.15	59.99	-11.85	-16.49
Medium P diet (.41% , 82 g/d)						
Feces	44.32	41.51	45.48	49.09	4.77	10.76
Milk	21.90	23.83	18.20	17.68	- 4.22	19.27
Urine	.75	1.36	1.36	1.54	.79	105.33
Total	66.66	66.71	65.04	68.31	1.65	2.48
High P diet (.55% ; 112 g/d)						
Feces	50.83	59.01	65.40	75.53	24.50	48.20
Milk	24.17	23.19	20.08	20.54	- 3.63	15.02
Urine	.79	3.37	4.91	4.36	3.57	451.90
Total	73.56	85.57	91.39	100.43	26.87	36.53

[a] During B 1 all cows consumed Medium P diet (.41%P, 82 g/d). Since values for B 1 through B 4 are least squares means, sums may differ slightly from total.

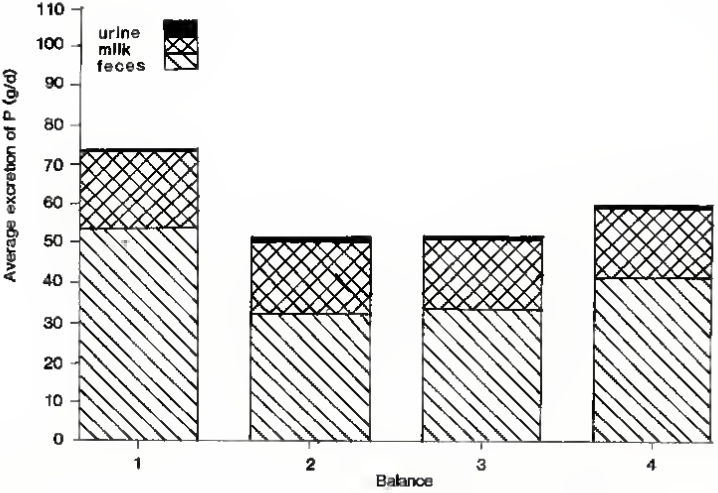
[b] Change g/d = B 4 - B 1.
Change % = (Change g/d)/B 1 * 100.

Cows assigned to 30% P diet (L) had a sharp decrease in excretion of P in feces during balance 2, but, excretion of P in feces gradually increased from balance 2 through balance 4 (Figure 4-4). Excretion of P in feces was 53.7 g/d when cows consumed 82 g of P/d, then dropped to 32.4, and gradually increased to 33.6 and 41.6 g/d during balances 2, 3,

Figure 4-3. Least squares mean daily excretion of phosphorus (g/d) by dairy cows during four intake--excretion balance studies. Each balance was 7 d in duration with 14 d between balances. Cows consumed 82 g of P/d.

Average excretion of P (g/d)
0
10
20
30
40
50
60
70
80
90
100
110
urine
milk
feces
1
2
3
4
Balance

Figure 4 4. Least squares mean daily excretion of phosphorus (g/d) by dairy cows during four intake—excretion balance studies. Each balance was 7 d in duration with 14 d between balances. Cows consumed 82 g of P/d during balance 1, and 60 g of P/d immediately thereafter through balance 4


urine
milk
feces
Average excretion of P (g/d)
110
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
Balance

and 4. During balances 1, 2, and 3 cows retained P (intake of P > excretion of P). Intake of P equalled excretion of P by balance 4. Excretion of P in feces decreased 22.7% from balance 1 to balance 4 when the difference between these balances was expressed as a percent of excretion during balance 1. There was a net decrease of 16.5% in total excretion of P from balance 1 to balance 4. A 26.8% decrease in quantity of P consumed was associated with a 22.7% decrease of P in feces and a 16.5% decrease in total excretion of P. Cows that were assigned to 56% P diet excreted 50.9 g of P/d in feces and had a total excretion of 73.6 g of P/d during balance 1 (Figure 4-5). Once cows began consuming the greater P diet (H), excretion of P in feces increased to 59.0, 66.4, and 75.5 g of P/d during balances 2, 3, and 4. Excretion of P in feces increased 24.5 g/d or 48.2% when compared to excretion of P during balance 1. The 46.6% increase in intake of P was associated with a 49.2% increase of P in feces and a 36.5% increase in total excretion of P.

Cows consuming M and H diets retained a portion of P consumed during all balances, but cows consuming L diet did not retain P during balance 4. Lack of retention of P by cows consuming L diet during balance 4 may have been a function of insufficient dietary P. Alternatively, one may argue that Ca was limiting in this diet, as increased concentration of P in the diet was confounded with increased concentration of Ca in the diet. It is possible that cows consuming L diet had adequate reserves and adequate dietary supply of P, and had no physiological need to retain P.

Quantity of P excreted in urine varied and was greatest for cows consuming diet H. Cows excreted < 9 ± 4 g of P/d in urine during

Fignre 4-5 Least squares mean daiiy excretion of phosphorus (g/d) by dairy cows dnring four intake--excretion balance stndies Each balance was 7 d in duration with 14 d between balances Cows consnmed 82 g af P/d during balance 1, and 112 g of P/d immadiately thereaftar through balance 4

Average excretion of P (g/d)
110
100
90
80
70
60
50
40
30
20
10
0
urine
milk
feces
1
2
3
4
Balance

balance 1 when all cows were consuming 82 g of P/d. Cows assigned to L or M diets excreted < 1.4 ± .4 g of P/d in urine during subsequent balances. Cows consuming diet H (112 g of P/d) increased excretion of P in urine to > 3.4 ± .4 g of P/d. Lomba et al. (1969) fed 41 different rations to 127 mature, nonpregnant, dry cows and an additional 14 rations to 35 mature lactating cows producing 11 to 20 kg of milk/d. They reported that excretion of P in feces and urine was variable and was not influenced by intake of P. Their findings may have resulted because of low intakes of P. Only four of the diets fed to lactating cows provided > 50 g of P/d. When low quantities of P were consumed, the ability of the animal to recycle and conserve P probably was variable and this probably accounted for highly variable results obtained.

Plasma samples taken weekly indicated all cows were adequate in status of P from weeks 1 through 8 of experiment. Fifty-six percent of the variation in concentration of P in plasma was partitioned to diet, cow(diet), week, and the interaction term of diet by week, but the latter two terms were not significant ($p = .14$ and $.24$). Cow(diet) was the error term used to test for significance of diet. Diet accounted for 19.3% of observed variation in concentration of P in plasma ($p = .02$). Least squares mean concentrations of P in plasma of cows fed the L, M, and H diets were 6.6 ± .5, 6.8 ± .4, and 8.3 ± .3 mg %. Concentrations of P in plasma (Table 4-4) were within the normal range of 3 to 8 mg % (Blood and Henderson, 1968).

Marker Technique

Indigestible markers were fed to estimate digestibility of DM by the marker technique and to compare estimates of excretion of P by this

technique with estimates obtained using total collection technique. Chromium at 750 mg of Cr/kg of DM, as Cr_2O_3, was included in concentrate portion of diets and ADL was native to feed ingredients. It was assumed that both markers were nonabsorbable, nondigestible, and not affected by digestive processes. Also, it was assumed that diets were mixed as formulated.

TABLE 4-4. LEAST SQUARES MEAN CONCENTRATIONS OF P IN PEASMA (Mg %) FROM WEEK 1 THROUGH WEEK 8, BY DIET AND WEEK.[a]

	Week[b]								
Diet[c]	1	2	3	4	5	6	7	8	SE
L	7.1	5.9	6.9	7.2	7.3	7.4	5.5	6.0	.9
M	7.0	7.2	7.6	6.2	7.9	6.5	6.5	6.5	.9
H	7.1	7.3	10.5	10.4	7.7	7.3	7.6	8.3	.7

[a] Variation was attributed to diet, cow(diet), week, diet*week. Model accounted for 56.5% of variation in concentration of P in plasma. SE = standard error of mean.

[b] Week number represents number of weeks consuming assigned diet when sample was obtained. Weeks 3 and 6 correspond to balances 2 and 3.

[c] Diet L = .30% P in diet DM
M = .41% P in diet DM
H = .56% P in diet DM

Two models were used to account for variation in digestibility values for DM and estimated excretion of P based on the three methods to determine digestibility. The first statistical model included diet, cow(diet), period, method, and interactions of diet by period, diet by method, and period by method (Appendix G, Table G-1). Interactions were not significant ($p > .10$) when partitioning variation in estimated excretion of P in feces. A reduced model eliminated interactions of diet by method and period by method and this was used to partition variation in digestibility of DM. Although interactions were significant ($p < .10$) for digestibility of DM the least squares mean

digestibiiities of OM for each method differed slightly when estimates from the two statistical models were compared. Inclusion of the interaction terms in partitioning digestibiiity of DM was associated with 5% of the observed variation

Digestibility of OM was affected by main effects of cow(diet), diet, period, method, and all interaction terms. Coefficients of digestibiiity calculated from total collection were greater than coefficients calculated using either marker source ($p < .01$). Digestibility of DM was 62.6 ± 1.4% when calculated from collection of feces but only 55.7 or 56.5 ± 1.4% when estimated by Cr and ADL. No differences were detected in estimated coefficients of digestion when Cr or ADL were used ($p > .10$). Determination of digestibiiity of DM by either Cr or ADL were equally precise, yet both methods underestimated actual digestibility of DM by 9.5%. Partial digestion of inert markers would lead to underestimation of ration digestibiiity. Fahey and Jung (1983) reviewed use of lignin as a marker. They concluded that underestimation of lignin resulted from partial digestion of lignin or from errors associated with the method used to determine lignin. Kotb and Luckey (1972) also reported that lignin was digested (< 0 up to 24%) in the GIT of ruminants. Chromium is not altered in the digestive tract. Since digestibility coefficients for Cr and ADL were similar, it does not appear that lignin was digested. Alternatively, it is possible that cottonseed hulls and concentrate represented greater portions of diets than formulated. If cottonseed hulls were included in the rations at a concentration greater than that formulated, concentration of P in TMRs would be less than the formulated value. If concentrate comprised

a greater proportion than that formulated, concentration of P would be greater than the formulated value.

Once digestibility of DM was estimated (Cr and ADL) and determined (total collection) excretion of P was estimated. Estimations of excretion of P in feces (g/d) were not affected by interactions of diet by method or diet by period. After interaction terms were removed the reduced model was used to obtain least squares mean estimated excretion of P for each method. Actual excretion of P was less than estimated excretion of P using either Cr or ADL marker techniques (49.7, 58.1, and 58.1 ± .7 g of P/d). This would be expected if digestibility of DM was underestimated because excretion of DM then would be overestimated. Since excretion of P was a function of excretion of DM, excretion of P also was overestimated by both markers.

Summary

Phosphorus was excreted in feces, milk, and urine. When intake of dry matter was restricted to 20 kg/d, cows consuming 82 g of P/d excreted 49.6, 21.9, and .9 g of P/d in feces, milk, and urine. When cows were switched to a diet containing only 60 g of P/d (.30% P; diet L) there was a 26.8% decrease in intake of P. After 9 wk of consuming diet L there was a 22.7% decrease in excretion of P in feces and a 16.6% decrease in total excretion of P. For each g of P decrease in intake/d, excretion of P in feces decreased .55 g/d. For cows that were switched to a diet high in concentration of P (diet H), intake of P increased 36.6% from 82 to 112 g of P/d. Total excretion of P increased 36.5%, while excretion of P in feces increased 49.2%. For each additional g of P consumed/d excretion of P in feces increased by .8 g/d. Potentially, excretion of P by cows consuming diets M or H can increase. Once cows

attain balance and no longer retain additional P the amount of P excreted will increase.

Chromium and ADL were equally precise at estimating digestibility of diet DM. These techniques underestimated actual digestibility of DM by 9.5% when compared to digestibility of DM determined by total collection of feces. Estimates of excretion of P (g/d) by marker technique were overestimated about 20% when compared to actual excretion of P (g/d) determined by total collection of feces.

CHAPTER 5
EFFECTS OF CONCENTRATION OF DIETARY PHOSPHORUS AND CALCIUM
ON VOLUNTARY INTAKE OF FEED AND PRODUCTION AND COMPOSITION OF MILK

Introduction

Concentration of P in feces was changed as concentration of P in feed changed (Chapter 4). Increasing the intake of P from 82 to 112 g/d (+48.8%) resulted in a parallel 48.6% increase in excretion of fecal P. Alternately, decreasing intake of P from 82 to 60 g/d (-26.9%) resulted in a 22.7% decrease in excretion of P. Voluntary intake of feed was restricted to 20 kg of DM/d in the previous experiment, thus it was not possible to evaluate if concentration of P in feed affected *ad libitum* intake of feed.

Few researchers have addressed effects of concentrations of dietary P and Ca on milk production of lactating dairy cows (Carstairs et al., 1981; Hibbs and Conrad, 1983, Kincaid et al., 1981, Steevens et al., 1971). Kincaid et al. (1981) assigned 48 Holstein cows at parturition to one of four dietary treatments in a 2 x 2 factorial arrangement. On a DM basis, concentration of P in feed was .30 or .54% and concentration of Ca was 1.0 or 1.7%. Feed intake and milk production were measured for 10-mo-postpartum. Average milk production was 29.5 kg/d over the entire lactation. Cows fed .54% P diets had increased yield of milk (2 kg/d) and increased intake of feed (2%) compared to cows fed .30% P diet. Steevens et al. (1971) assigned 48 lactating cows to one of three

diets at 3-wk-postpartum. Concentrations of P were .4%, .6%, or .8% of diet DM with Ca:P ratios of 3:1, 3:1, or 1.5:1. Cows were fed assigned diets throughout the entire lactation and 16 wk into the next lactation. The DM intake, milk production and milk composition were not affected by diet fed. Cows consuming diets containing .6% P had greater concentration of P in serum (7.4 vs 6.3 mg %). Although these values differed, differences may not be important biologically since concentrations for all cows on all diets were within normal range expected. Carstairs et al. (1981) evaluated effect of dietary concentration of P and energy intake on milk yield and composition of lactating dairy cows. Average yield of milk was 22.5 kg/d. Concentration of P (.4% or .5% of diet DM) did not affect protein or fat concentration of milk. Cows consuming the .4% P diet produced 1.9 kg/d more milk during weeks 5 through 12 of lactation than cows consuming the .5% P diet. No differences in yield of milk were apparent during the first 5 wk of lactation. These authors concluded that P should not exceed levels recommended by the NRC (1978) (.32 to .40% of diet DM, based on animal weight and milk production).

Do dietary concentrations of P between .30 and .54% of diet DM and Ca at .60 or .97% of diet DM affect voluntary intake of feed or production of milk by lactating dairy cows producing > 20 kg of milk/d? The objectives of this experiment were to determine effects of concentrations of dietary P and Ca on voluntary intake of feed and on production and composition of milk.

Materials and Methods

Twenty-four (13 multiparous, 11 primiparous) lactating Holstein cows were used in a partially balanced randomized incomplete block design. Cows were more than 70 d in lactation at the beginning of the experiment. It was assumed that intake of DM had peaked by the first data collection period. Therefore, effects of diet were not confounded with increased milk production during early lactation. Three dietary concentrations of P and two dietary concentrations of Ca were evaluated (Table 5-1). Actual concentrations of P in diets were .33, .43, and .54% of diet DM. This represented 83% (deficient), 109% (adequate), and 135% (excess) of current recommendations for high producing cows (.41% of diet DM as P; NRC, 1989). Concentrations of Ca were .60 and .97% of diet DM representing 100% and 162% of current recommended concentrations of Ca (NRC, 1989).

TABLE 5-1. CALCIUM-PHOSPHORUS RATIOS OF DIETS CONTAINING DIFFERENT AMOUNTS OF CA AND P.

Concentration of Ca (% DM)	Concentration of P (% DM)		
	.33	.43	.54
.60	1.8 (1)*	1.4 (2)	1.1 (3)
.97	2.9 (4)	2.3 (5)	1.8 (6)

* Diet number in parenthesis.

Cows were housed in a freestall barn with access to sand exercise lots. Cows were fed TMR individually twice daily using Calan gates at 0900 and 1430 h. Fresh water was available *ad libitum*. Basal diet (Table 5-2) contained .33% P and .60% Ca on a DM basis. The two greater concentrations of P were obtained by addition of monoammonium phosphate and a simultaneous reduction in quantity of urea to balance the nitrogen

content of feed. Higher concentration of Ca (.97%) was achieved by addition of feedgrade $CaCO_3$.

TABLE 5-2. DRY MATTER COMPOSITION OF BASAL DIET.

Ingredient	% of diet DM
Ground corn	49.15
Corn silage	17.98
Cottonseed hulls	15.41
Soybean meal (48%)	13.49
Urea	1.73
$CaCO_3$	1.02
Trace mineral salt	.84
Magnesium oxide	.32
Monoammonium phosphate	.07
Total	100.00

Cows were trained to use Calan gates during a 12 d adjustment period. At that time, cows consumed the basal diet (.33% P; .5% Ca) ad libitum. Thereafter they were assigned randomly to three of six dietary treatments and each diet was consumed during one of three periods. Each cow consumed at least one diet with each concentration of Ca (Table 5-3). Periods were 28 d in duration with feed intake and milk production quantified during the last 7 d. All 24 cows provided data during all three periods.

Concentrates were mixed every 3 to 4 wk. Samples of concentrate were taken once weekly and analyzed for P and Ca. Corn silage was sampled three times weekly for determination of DM and concentrations of P and Ca. Cottonseed hulls were sampled biweekly for determination of DM and concentrations of P and Ca. During the last 7 d of each period TMRs were sampled for determination of DM and nutrient composition. Two composite samples per diet were made for each period. Samples of TMRs from d 22 through 25 and from d 26 through 28 were pooled for each diet in each period and represented the two composite samples. Each

TABLE 5-3 DIET ASSIGNMENT FOR EACH COW BY PERIOD DURING FEEDING STUDY.

	Period		
Cow	I	II	III
1113	1	3	4
1164	5	1	2
1207	4	6	1
1223	2	1	5
1239	4	3	6
1279	6	5	3
1291	5	4	2
1314	6	2	3
1319	2	5	1
1363	1	6	4
1397	4	2	3
1419	2	6	5
1419	1	4	3
1453	4	1	6
1461	5	2	1
1464	6	3	2
1469	1	6	6
1477	3	6	2
1490	6	4	1
1492	3	1	4
4671	5	3	4
4675	3	2	6
6331	3	6	6
9832	2	4	5

composite sample of TMR was analyzed for concentrations of P and Ca (Table 5-4). A sample of each diet pooled across periods was sent to the New York Forage Testing Laboratory for analysis of nutrient composition (Table 5-5). Orts were weighed and sampled daily (d 23 through 28, and d 1 of the next period) for determination of DM. Intake of DM was calculated as quantity of DM offered minus that refused. It was assumed that the concentration of nutrients in orts was the same as the concentration of nutrients in feed offered and that animals did not sort feed.

Milk weights were recorded at each milking by automatic DeLaval system. Milk yield for am and pm milkings was averaged for the last 7 d of each period. Composition of milk was determined on samples obtained

TABLE 5 4. CONCENTRATION OF PHOSPHORUS AND CALCIUM IN TOTAL MIXED RATIONS, BY PERIOD.

Ration	Period I	Period II	Period III
	Phosphorus, % dry matter		
1[a]	.31	.33	.33
	.34	.33	.34
2	.45	.41	.41
	.46	.44	.41
3	.52	.57	.52
	.58	.53	.51
4	.31	.36	.34
	.32	.35	.29
5	.43	.44	.41
	.41	.42	.41
6	.52	.56	.52
	.57	.58	.51
	Calcium, % dry matter		
1	.61	.54	.58
	.55	.55	.58
2	.62	.64	.58
	.62	.51	.71
3	.70	.59	.63
	.56	.57	.59
4	.82	1.18	.74
	.84	.91	.91
5	1.18	1.18	.99
	.92	1.03	1.01
6	.85	.87	.91
	1.13	.92	.56

[a] Means obtained from chemical analyses of total mixed ration samples composited d 22 through 25 and for d 26 through 28.

TABLE 5-5 NUTRIENT COMPOSITION OF DIETS FROM SAMPLES ANALYZED AT NEW YORK DAIRY HERD IMPROVEMENT FORAGE TESTING LABORATORY.[a]

Nutrient[b]	Diet					
	1	2	3	4	5	6
	percent of dry matter					
Crude Protein	16.1	15.4	15.4	15.6	13.5	14.7
ADF	23.1	22.6	21.2	21.0	24.5	22.4
NDF	35.9	36.0	36.4	35.5	39.9	36.1
TDN	75	76	77	77	74	76
NE_l	.70	.71	.72	.72	.69	.71
Calcium	.60	.61	.63	.95	.79	.91
Phosphorus	.33	.42	.48	.33	.37	.51
Potassium	1.01	1.03	1.00	.99	1.02	1.01
Magnesium	.41	.42	.37	.41	.43	.41
Sodium	.49	.49	.52	.56	.56	.54
	parts per million					
Iron	496	593	421	433	467	513
Zinc	42	37	36	36	37	37
Copper	21	23	15	22	27	15
Manganese	128	139	84	130	179	84
Molybdenum	1.8	1.9	1.5	1.5	1.5	1.8

[a] Ithaca, New York.

[b] ADF = Acid detergent fiber (%).
NDF = Neutral detergent fiber (%).
TDN = Total digestible nutrients (%).
NE_l = Net energy for lactation (Mcal/kg).

from the last six milkings (3 d) of each period. Percent of milk as fat, protein, lactose, solids-non-fat, and the somatic cell count were determined (Dairy Herd Improvement Processing Lab, Blacksburg, VA).

Average daily fat percent was determined by the summation of quantity of fat produced divided by the summation of quantity of milk produced for am and pm milkings. Similar calculations were made for protein, lactose, and solids-non-fat. Average milk production was the summation of milk produced during d 22 through 28 divided by number of d. Average production was calculated for am and pm milkings.

Samples of whole blood were obtained from cows once weekly after am milking but before morning feeding. Samples were collected from the

tail vein into heparinized tubes. Samples were stored on ice, centrifuged within 1 h at 2,500 (770 x G) rpm for 15 min, plasma aliquoted into 15 ml opaque polypropylene tubes, capped, and stored on ice during transportation to the laboratory. Samples with hemolysis were discarded and replacement samples were obtained the next d. Plasma was precipitated with 10% TCA and analyzed for concentration of P, as described previously (Chapter 3).

Statistical Analyses

Data were analyzed by method of least squares analysis of variance using the General Linear Models procedure (SAS, 1985). Variation in dependent variables was attributed to age, cow(age), period, diet, age*period, and age*diet. Interaction of age*diet was not significant ($p > .35$) and was removed from the model. Cow(age) was error term used to test for dietary significance.

Results and Discussion

Average daily intake of DM adjusted for age and dietary treatments, decreased from 24.7 ± .4 kg/d in period I to 23.9 ± .4 kg/d in period III. Least squares analysis of variance tables are in Appendix H. Intake of DM was not different for ratios of Ca:P between 1.1:1 and 2.9:1 (Table 5-9). Concentrations of P or Ca in diets did not affect intake of DM, but there was a nonsignificant trend for intake of DM to increase as concentration of P decreased. This was opposite results of Kincaid et al. (1981). They reported that cows fed a diet of .3% P and 1.0% Ca consumed 7% less feed than cows fed a diet containing .54% P and 1.0 or 1.7% Ca. Concentrations of Ca in diets fed by Kincaid et al. (1981) were greater than those in diets fed during this experiment. The high

TABLE 5-6. LEAST SQUARES ANALYSIS OF VARIANCE FOR DEPENDENT VARIABLES PROBABILITY VALUES, COEFFICIENTS OF VARIATION (CV), AND LEAST SQUARES (LS) MEANS FOR MODELS ANALYZED.

		Dependent variable[a]							
Source	DF[b]	DM	MY	3.5% MY	FAT%	FAT% MY	PRO%	PRO% MY	BLD
Age	1	.31	< .01	.03	.03	.11	.09	< .01	.04
Cow(Age)	22	< .01	< .01	< .01	< .01	< .01	< .01	< .01	.02
Period	2	.01	< .01	< .01	< .01	.24	< .01	< .01	.65
Treatment									
Phosphorus(P)	2	.77	.09	.37	.37	.56	.74	.72	.15
.33 vs .43 & .54	1	.54	.07	.17	.17	.97	.88	.46	.10
.43 vs .54	1	.73	.25	.85	.85	.29	.46	.75	.26
Calclnm(Ca)	1	.47	.02	.03	.03	.66	< .01	.33	.71
P*Ca	2	.45	.57	.72	.72	.95	.78	.87	.61
Age*Period	2	.79	.03	.02	.02	.24	.23	.66	.59
CV		8.0	5.4	7.4	7.4	7.3	2.3	1.1	14.3
R^{2c}		.80	.96	.91	.91	.80	.94	.94	.64
LS Means									
Phosphorus	33	24.1	22.8	24.0	22.2	3.9	3.5	4.9	7.0
	43	23.8	22.3	23.3	21.6	3.8	3.5	4.9	7.7
	54	23.5	21.8	23.2	21.5	3.9	3.5	4.9	7.3
SE		.4	.3	.4	.4	.1	.02	.01	.2
Calclum	60	23.7	22.7	24.0	22.2	3.9	3.5	4.9	7.4
	97	24.0	21.9	23.0	21.3	3.9	3.5	4.9	7.3
SE		.3	.2	.3	.3	.1	.01	.01	.2

[a] Dependent variables are: DM = average daily intake of dry matter, MY = actual milk yield; 3.5% = 3.5% fat corrected milk; 4.0% = 4.0% fat corrected milk; FAT% = percent fat, PRO% = percent protein, LAC% = percent lactose, BLD = concentration of P in plasma as mg %.

[b] Degrees of freedom associated with source, DF.

[c] R^2. Percent of variation in dependent variable explained by the model. Calculated as model sum of squares divided by total sum of squares.

concentrations of Ca may have bound part of P as mono or dicalcium phosphate rendering it unavailable. Hence, increased concentration of P would have increased available P for animal use.

When adjusted for age, period, and concentration of Ca in diet, concentration of P in diet affected yield of milk ($p < .10$). Cows consuming a diet of .33% P produced more milk ($p < .07$) than cows consuming diets with higher (.43 and .54%) concentrations of P (22.8 vs 22.3, 21.8 kg of milk/d). Actual yield of milk was the same for cows fed .43 or .54% P diets ($p < .25$). No differences were detected for yield of 3.5 or 4.0% FCM due to concentration of P in diet. These results indicated that concentrations of P in diets greater than .33% were not beneficial when milk production was less than 23 kg/d. Carstairs et al. (1991) reported that cows fed a diet containing .4% P (> concentration then recommended by NRC, 1979) produced 1.8 kg/d more milk than cows fed a diet containing .5% P. Percentages of fat, protein, and lactose were not affected by concentration of P in diet when results were adjusted for age, period, and concentration of Ca. This agreed with Carstairs et al. (1991) who reported that concentration of dietary P at .4 or .5% of diet DM did not alter protein or fat percent of milk.

Milk yield, but not intake of DM, differed for the cows fed the two dietary concentrations of Ca. Cows fed diets containing .6% Ca produced more milk ($p < .02$) than cows fed diets containing .97% Ca (22.7 vs 21.9 ± .2 kg/d). Kincaid et al. (1991) reported no differences in milk yield due to concentration of Ca in diet when Ca was fed at 1.0 and 1.7% of diet DM. The lower concentration of Ca was greater than .97% used in

this experiment and greater than currently recommended concentration of .60%.

Summary

Voluntary intake of DM and FCM of cows did not differ when they were fed TMRs containing Ca:P ratios of 1.1:1 to 2.9:1 or three dietary concentrations of P (.33, .43, and .54% of DM). The trend was for greater intake of DM at .33% P. Production of 3.5% FCM and 4.0% FCM were 4.8% ($p < .03$) and 4.7% ($p < .03$) greater by cows fed a .60% Ca diet when compared to cows fed a .97% Ca diet. Feeding diets containing concentrations of P greater than NRC (1989) recommended concentrations (.43 and .54%) did not improve voluntary intake of feed or production of milk.

Dietary concentration of Ca at .97% of diet DM was detrimental to production of 3.5% and 4.0% FCM.

CHAPTER 6
DISAPPEARANCE OF PHOSPHORUS BOUND TO PHYTATE
FROM FEEDSTUFFS INCUBATED IN VITRO

Introduction

High producing lactating dairy cows require tremendous intakes of energy, protein, vitamins, minerals, and water to maintain lactation. In such cows, intake of concentrate generally exceeds intake of roughage (DM basis). The concentrate portion usually consists of cereal grains, grain by-products, and protein supplements. In these ingredients, concentrations of P bound to phytate typically range from 50 to 75% of total dietary P (Common, 1940, Nelson et al., 1968a). If P bound to phytate were unavailable to animals for biological processes, concentration of P in feeds would need to be discounted due to concentration of P bound to phytate. This is especially important since P bound to phytate represents such a large percent of the total P in concentrates and concentrates contribute 60 to 90% of total dietary P for dairy cattle.

In a review of the utilization of P bound to phytate by animals, Nelson (1967) concluded that "there is still no general agreement on the extent to which different species of animals at various ages utilize phytate P". (p. 862). Although 20 yr have past, this statement remains essentially true. Most research evaluating utilization of P bound to phytate has been conducted using poultry. Although some researchers

reported that natural phytate was a poor source of P for poultry (Gillis et al., 1949; Heuser et al., 1943; McGinnis et al, 1944) other researchers reported almost complete availability of P in finely ground wheat flour (Sieburth et al, 1952) or greater availability of P in heat treated than nonheat-treated concentrates (McCance and Widdowson, 1944, Summers et al., 1967). Temperton et al. (1965a, 1965b, 1965c) reported that poultry which had greater demands for P (i.e., growing chicks and laying hens) had greater efficiency of utilization of organic vs synthetic sources of P than did poultry which had lesser demands for P (nonlaying and nongrowing). Differences in results may be attributed to source of phytate used (natural vs. synthetic), size of feed particles, criteria of response (gain of weight, development of bone, or concentration of P in plasma or serum), age of animals, status of production, or concentrations of Ca and vitamin D in the diet.

Differences in availability of P bound to phytate by nonruminants from natural vs synthetic sources may have resulted because of presence or absence of phytase. Phytase is the enzyme responsible for hydrolyzing P bound to phytate to yield P_i and inositol ring (McCance and Widdowson, 1944, Nelson et al., 1968b, Pointillart et al., 1987, Summers et al., 1967).

Few studies with cattle have evaluated the availability of P bound to phytate. When Mathur (1953) fed dairy cows a highly reactive limestone, 36 to 51% of the total P bound to phytate was excreted in feces. He concluded that this resulted because of precipitation of phytic acid as calcium phytate. Clark et al. (1966) examined availability of P bound to phytate in high producing dairy cows during early lactation when intakes of grain (18.3 to 21.5 kg of DM/d) and

concentrations of Ca (.6 to .9% of DM) were high. Calcium was supplemented from calcite flour, aregonite, and albecar. Ninety-four to 99% of P bound to phytate was hydrolyzed to P_i. It was not determined where in the digestive tract phytate was hydrolyzed.

Nelson et al. (1976) evaluated hydrolysis of P bound to phytate from natural sources in Holstein calves (56 d of age) and steers (9 mo of age). Calves were fed a commercial milk replacer twice daily from 3 d through 45 d of age. Additionally, they were fed a concentrate diet containing .5% P of which 58% of P was bound to phytate. At 56 d of age, calves were placed in metabolism crates for collection of excreta (7 d preliminary, 7 d collection). While in metabolism crates calves consumed 1.5 kg of concentrate/d. Trace amounts of P bound to phytate were recovered in feces of young calves but greater than 99% was hydrolyzed. Intake and excretion of P bound to phytate was 20 ± 1.8 g/d and .06 ± .014 g/d. Two other trials were conducted with steers. In one trial six steers were fed 1 kg of concentrate twice daily which contained .47% P with 49% of total P bound to phytate. No P bound to phytate was recovered in feces from these steers. An additional trial was conducted to determine concentration of P bound to phytate in feedstuffs removed from various segments of the GIT. Three steers were fed 3 kg of concentrate/d (.50% P, 58% bound to phytate). Steers were removed from pasture, fed the concentrate diet for 7 d, fasted 18 h, and then slaughtered. Contents of the rumen, abomasum, and small and large intestines were taken immediately and stored at -10 C until dried for analysis. No P bound to phytate was recovered from contents of digesta from any compartment. Reid et al. (1947) reported that hydrolysis of P bound to phytate *in vivo* occurred in less than 8 h in sheep. Hence,

lack of recovery of P bound to phytate may have been due to the long fast imposed by Nelson et al. (1976) before recovery of contents of the digestive tract.

The objectives of this experiment were 1) to determine concentration of P bound to phytate in by-product feeds commonly fed to dairy cattle, and 2) to determine solubility of P bound to phytate *in vitro* and hydrolysis of P bound to phytate *in vitro* and *in vivo*. By-product feeds evaluated *in vitro* were cottonseed meal, dried distillers grain, hominy, rice bran, soybean meal and wheat middlings.

Materials and Methods

Samples of feedstuffs were obtained from a commercial feedmill (United Feed Cooperative, Okeechobee, FL). Cottonseed meal was sieved through a 2 mm screen to remove any remaining lint. All other feedstuffs were small enough to pass through the 2 mm sieve.

A lactating dairy cow fitted with a rumen cannula provided rumen fluid. Rumen fluid was obtained after the morning milking and prior to morning feeding. The cow consumed a diet of corn silage and concentrate mix (55:45 DM basis). Concentrate portion included ground corn, soybean meal, and occasionally dried distillers grain or whole cottonseed. Rumen fluid was obtained on five separate occasions (batches) and incubated with two feedstuffs per batch. Rumen fluid was collected, placed in a preheated thermos jug, and transported immediately to the laboratory for processing. Rumen fluid and McDougall buffer were mixed (1 to 4), as described (Tilley and Terry, 1963). After mixing, CO_2 was bubbled through the solution to bring the pH to between 6.96 to 6.99. Then 150 ml of solution was dispensed into 250 ml Erlenmeyer flasks containing 3 g (dry basis) of a feedstuff. Flasks and substrates were

in triplicate. Solution and feedstuff were mixed by gentle swirling. Air in flasks was displaced with CO_2, flasks were stopped with one-way valves and placed in a 39 C incubator. Flasks were removed after 1, 2, 4, 6, 8, 10, 12, and 24 h of incubation. A total of 24 flasks per feedstuff were incubated with rumen fluid/McDougall buffer for each batch.

At each time interval when flasks were removed from incubator, remaining flasks were swirled gently. A set of samples (in triplicate) also were incubated for 24 h in McDougall buffer to determine loss of P bound to phytate attributed to activity of phytase in feedstuff. Flasks were unstoppered, pH was recorded, and 12M HCl was added dropwise to kill bacteria and stop reaction of phytase (pH < 2). Contents were transferred to 50 ml nalgene tubes, centrifuged (2,500 rpm; 1020 x G, 15 min), and filtered through coarse filter paper (Whatman 41). Solids were rinsed three-times with DI and finally with acetone. After final rinsing, particulate matter was rolled in filter paper, placed in 50 ml nalgene tube, and dried overnight at 50 C. For the 1, 2, 8, 12, and 24 h incubations, 10 ml of filtrate were used for determination of P bound to phytate. Duplicate samples of McDougall buffer and rumen fluid solution were used to quantify presence of P bound to phytate in the absence of cereal grain (blank). Value of P bound to phytate in filtrate was adjusted for value of P bound to phytate in blank tubes.

A laboratory standard (sodium phytate from corn) was used as a standard for the phytate procedure. Phytate was extracted by the method of Earley (1944) with one modification: 1.2% HCl containing 10% Na_2HSO_4 was added (20 ml/g dried sample) and tubes were capped and placed on shaker for 6 h at room temperature. (Note: the method of Earley (1944)

called for 2 h of shaking, there were no differences in extraction of P bound to phytate if samples were shaken for 2, 4, or 6 h). Ten ml of extract were placed in each of two nalgene tubes. Phosphorus bound to phytate was precipitated with 6 ml of 4% $FeCl_3$ in 5% HCl containing 5% Na_2SO_4 (Earley and DeTurk, 1944). Tubes were heated in a 100 C oven for 60 min and then cooled on ice. A white precipitate formed. This contained di- to hexaphosphates (de Boland et al., 1975). Tubes were centrifuged (2,500 rpm, 15 min, 1020xG), supernatants decanted, and precipitates rinsed with 6% HCl containing 5% Na_2SO_4. Centrifugation and rinsing were repeated three times. Precipitate was transferred with DI into 30 ml pyrex beakers. Beakers were placed in a 100 C oven until dry, then were ashed at 550 C for 4 h. Concentration of P was determined by method of Harris and Popat (1954), as described previously (Chapter 3).

Digestibility of P bound to phytate was determined in vivo. Fecal samples from the fourth balance of the digestion study (Chapter 4) were analyzed for concentration of P bound to phytate. Digestibility of phytate bound P was calculated as the difference between quantities of P bound to phytate in feed and P bound to phytate in feces, divided by P bound to phytate in feed.

Phytate bound P was extracted from 3.0 g of dried feces using 40 ml 1.2% HCl containing 10% Na_2SO_4. Determination of phytate was as described for feedstuffs. Also, lignin and Cr were used as indigestible markers to determine digestibility of P bound to phytate (Kane et al., 1953). Concentrations of ADL, Cr, and P bound to phytate in diets fed were 6.0, .075, and .17% of diet DM. Additionally, 20 fecal samples from the December, 1986 collection for the field study were selected

randomly from NF-3 and NF-4 (Chapter 3). Assuming a concentrate:forage ratio of 80:20, on a DM basis, mean concentration of P bound to phytate was .38%. Lignin comprised 6.1% of diet DM for NF-3 and 5.0% of diet DM for NF-4.

Results and Discussion

Concentrations of P bound to phytate for feedstuffs sampled ranged from 1,900 to 11,150 mg/kg of DM (Table 6-1). Concentration of total P ranged from 2,980 to 15,780 mg/kg of DM. Expressed as a percent of total P, 32.0 to 80.7% of P was bound to phytate. Dried distillers grain had the lowest concentration of P bound to phytate (32.0%), whereas other ingredients analyzed had greater than 55% of P bound to phytate. These were similar to values published (Common, 1940; Nelson et al. 1968a). Common (1940) reported that P bound to phytate, for five ingredients as a percent of total P, were as follows: wheat (77.6%), oats (74.3%), corn meal (78.3%), bran (77.7%), and extracted soybean

TABLE 6-1. CONCENTRATION OF TOTAL-P AND P BOUND TO PHYTATE (PHY-P) IN COMMON FEEDSTUFFS FOR DAIRY COWS.

FEEDSTUFF	N	TOTAL P	PHY-P	PHY-P	NELSON*
---	---	---PPM---		% Total P---	
Cottonseed meal	2	12220	8480	69.4	70
Dried distillers grain	2	9990	2880	32.0	43
Ground corn	3	2980	1900	63.8	66
Nommy	3	6380	4690	73.4	na[b]
Peanut meal	2	6650	3800	57.1	na
Nica bran	2	15780	12560	80.7	86
Soybean meal	2	7320	5020	68.6	56
Wheat Middlings	3	13400	10480	78.2	74

* Reported values of P bound to phytate as % of total P by Nelson et al. (1968). Standard deviations for corn and soybean meal were 7%.

[b] na = data not reported.

N = number of different samples analyzed in triplicate.

(53.9%). Results from lab analyses in this experiment were similar to those of Nelson et al. (1968a) for percent of total P as P bound to phytate: cottonseed meal, 70%, ground corn, 66%, rice bran, 86%, and wheat middlings, 74%. Nelson et al. (1968a) reported standard deviations of 7 to 9% for most samples analyzed. Concentrations of total P bound to phytate were much less for forages. Less than 01% of P was bound to phytate in alfalfa hay, bermuda, brome, orchard, or fescue grass (Nelson et al., 1976).

Amount of P bound to phytate was quantified after *in vitro* incubation with rumen fluid/McDougall buffer for up to 24 h. The first statistical model for analysis of data was a split split plot design with true replication (Table 6-2). Batch of rumen fluid used was not significant ($p < .49$) and was dropped from the model. The reduced model

TABLE 6-2. LEAST SQUARES ANALYSIS OF VARIANCE OF VARIATION IN CONCENTRATION OF P BOUND TO PHYTATE IN FEED INGREDIENTS AFTER VARYING INCUBATION TIMES IN BATCHES OF ARTIFICIAL RUMEN FLUID/MCDOUGALL BUFFER.

Source	DF	Type III SS	F	PR
Batch[a] (B)	4	21805.6852	1.00	.4845
Feed(B)	5	27181.5337	210.83	.0001
Hour (Hr)	7	69424.7957	384.64	.0001
Hr*Feed(B)	57	4424.5103	30.10	.0001
Error	397	10236.5265		

[a] 5 batches of rumen fluid, 2 feeds per batch. Samples removed after 1, 2, 4, 6, 8 ,10, 12, and 24 h of incubation.

permitted separation of components of the combined overall error term (Table 6-3). Mean square for error (σ^2_{rep}) was 1.83 and estimated sample variance (σ^2_{samp}) was 31.7 using a k value of 2.4 (471/198). Based on estimates of σ^2_{samp} and σ^2_{rep} it was possible to estimate variance of the mean $\sigma^2_{\bar{y}}$. Greatest reduction in variation would occur by increasing number of samples per feed*hr combination. As number of samples

TABLE 6-3. LEAST SQUARES ANALYSIS OF VARIANCE OF VARIATION IN CONCENTRATION OF P BOUND TO PHYTATE IN FEED INGREDIENTS AFTER VARYING INCUBATION TIMES IN AN ARTIFICIAL RUMEN ENVIRONMENT.

Source	DF	Type III SS	F	PR
Feed[a] (F)	9	53521.9497	76.33	.0001
Hour (Hr)	7	68893.2576	126.32	.0001
F*Hr	57	43691.7212	9.84	.0001
Sample(F*Hr)	125	9739.7465	42.57	.0001
Repisample(F*Hr)]	272	200374.7037		

[a] 10 feed-batch combinations of artificial rumen environment. Samples removed after 1, 2, 4, 6, 9, 10, 12, and 24 h of incubation.

increased from 1 to 4, estimates of σ^2_y decreased from 33.5 to 17.7 to 12.4 to 9.8. If these samples were replicated twice, estimates of σ^2_y would be 32.6, 16.9, 11.5, and 8.9. Based on availability of equipment, triplicate samples were exposed to *in vitro* fermentations and replicates were made prior to determination of phytate. For this procedure σ^2_y was 11.5.

Although effects of batch of rumen fluid on hydrolysis of phytate bound P did not differ when the same ingredients were incubated in more than one batch, batches were considered different for analytical purposes. Least squares mean values for disappearance of P bound to phytate were expressed as percent of total P initially bound to phytate (Table 6-4). As duration of incubation increased from 1 to 24 h percent disappearance of P bound to phytate increased for most feedstuffs. Results for cottonseed meal were least consistent. Percent disappearance of P bound to phytate for 1, 2, 4, 6, and 9 h were 35.2, 49.7, 17.9, 57.0, and 39.6%. Nonuniform mixing of cottonseed meal prior to weighing ingredient into flask, or inadequate or too infrequent swirling of fluid and ingredient may account, in part, for variation observed. The slower release of P bound to phytate also may have

TABLE 6-4. LEAST SQUARES MEAN VALUES FOR DISAPPEARANCE OF P BOUND TO PHYTATE (EXPRESSED AS PERCENT OF INITIAL P BOUND TO PHYTATE) BY DURATION OF INCUBATION IN VITRO (HR) AND INGREDIENT[a]

Hr	WM1[b]	WM2	WM3	WM4	RB1	RB2	HOM	SBM	DDG	CSM
1	----	----	----	66.4	----	85.1	90.6	----	----	35.2
2	35.9	84.5	79.9	60.7	80.7	85.3	62.5	36.3	71.3	48.7
4	57.5	87.8	83.0	59.6	77.7	78.4	63.6	50.2	96.1	17.8
6	66.1	94.9	75.1	97.8	92.6	82.6	99.0	50.2	95.4	57.0
8	99.7	95.9	88.4	90.0	97.0	90.8	98.6	99.5	95.4	39.6
10	99.9	96.0	96.9	93.6	98.8	94.4	98.6	99.9	98.9	66.5
12	99.9	98.1	98.9	96.5	99.6	98.1	99.4	99.9	98.7	71.5
24	100.0	99.8	99.9	99.9	99.9	99.8	99.8	100.0	99.3	93.9
24[c]	----	58.6	----	40.6	----	57.3	40.1	----	81.0	14.7

[a] Ingredients: wheat middlings, WM; rice bran, RB; hominy, HOM, soybean meal, SBM, dried distillers grain, DDG; cottonseed meal, CSM. Numbers after ingredient abbreviations represent separate incubations of similar ingredients. Incubation media was 1 part rumen fluid:4 parts McDougall buffer (Tilley and Terry, 1963).

[b] ---- no data available.

[c] Sample incubated in McDougall buffer alone for 24 hr.

resulted from high protein content and chemical changes induced during processing. Fontaine et al. (1946) reported that in the presence of high concentration of protein the P bound to phytate was less available for animal use. Slower release of P bound to phytate also was evident for soybean meal at 8 h (50.2%). Chemical bonds formed between protein and P bound to phytate must be broken before phytase can liberate P_i from phytate (Fontaine et al., 1946).

The rapid loss of P bound to phytate for most of the feed ingredients evaluated (>85% by 8 h; Table 6-4) agreed with in vivo data in sheep (Reid et al., 1947). Four sheep were fed one of three diets for several d prior to slaughter. At slaughter, samples of contents from the rumen, reticulum, abomasum and throughout the intestine were removed and dropped into boiling DI to prevent further action by

phytase. Two of these sheep consumed the same diet (.7% P_2O_5 of diet DM) and were slaughtered 1 or 8 h postfeeding. The P bound to phytate was quantified in contents of rumen and reticulum from the sheep slaughtered 1 and 8 h postfeeding. No phytate bound P was detected in the GIT contents of sheep slaughtered 8 h postfeeding. The authors concluded that there had been complete hydrolysis of P boued to phytate. As digesta fluids in compartments of the GIT were not analyzed, it is possible that P bound to phytate was merely liberated from ingredient aed remained unhydrolyzed in solution.

Hydrolysis of P bound to phytate in artificial rumen solution was quantified in duplicate samples of filtrate from current in vitro studies. Samples were analyzed for content of P boued to phytate after 1, 2, 6, 12, and 24 h of incubation and after 24 h incubation in McDougall buffer alone. Least squares mean values for concentration of P bound to phytate in filtrate were expressed as mg/kg of original DM (Table 6-5). Variations due to sample and replication(sample) were σ^2_{samp}=259403 and σ^2_{rep}=89055. Total recovery of P bound to phytate (that remaining in solid residue plus that in filtrate) was greater than initial values for wheat middling (WM4) at 1 and 2 h, rice bran at 1, 2, and 6 h, and cottonseed meal at 1 h. These discrepancies resulted from standard errors (130 to 250 mg of P/kg of DM) associated with determination of concentration of P bound to phytate in filtrate and assay variation. After 6 h of incubation, concentration of P bound to phytate in solution decreased over time for all feedstuffs. By h 12, virtually all P boued to phytate (> 95%) had been hydrolyzed.

Disappearance of P bound to phytate from feed ingredients aed from incubation media in vitro suggested high availability of phytate bound

TABLE 6-5. LEAST SQUARES MEANS FOR CONCENTRATIONS OF P BOUND TO PHYTATE IN SOLUTION FROM RUMEN IN VITRO INCURATIONS[a]. VALUES EXPRESSED AS PARTS PER MILLION OF P PER GRAM OF DRY MATTER IN IN VITRO FERMENTATION FLASK BY DURATION OF INCURATION (HR) AND FEED[b]

HR	WM2[b]	WM4	RB2	HOM	DDG	CSM
1	--[c]	10060	17710	1620	----	4750
2	8240	7530	16030	950	1320	4290
6	5160	7540	13082	180	920	4720
12	--	200	6	6	----	590
24	40	80	0	0	70	0

[a] Incubation media consisted of 1 part rumen fluid:4 parts McDougall buffer (Tilley and Terry, 1963)

[b] Ingredients: wheat middlings, WM, rice bran, RB, hominy, HOM, dried distillers grain, DDG, cottonseed meal, CSM. Numbers after ingredient abbreviations represent separate incubations of similar ingredients.

[c] -- no data available

P. Fecal samples were analyzed for P bound to phytate from the fourth collection period described previously (Chapter 4). Concentration of P bound to phytate in feed was 17% of diet DM. Trace amounts of P bound to phytate in feces were recovered. Digestibility of phytate bound P was greater than 99.9%, based on estimates of intake and excretion. When Cr was used as an indigestible marker, digestibility of phytate bound P was greater than 99.9%. Slightly lower values (94 to 98%) were obtained when lignin was the indigestible marker. The lower values obtained from use of lignin as an indigestible marker may have occurred because less than 100% of lignin was recovered from contents of the GIT. When lignin is underrecovered, digestibility of feedstuff is underestimated. These results were similar to those of Clark et al. (1966). In their study, cows consumed 22% of diet DM as P bound to phytate. They reported that greater than 98% of dietary P bound to phytate was hydrolyzed in lactating dairy cattle.

Twenty fecal samples from cows fed diets with high concentration of P bound to phytate (.38% of diet DM) were analyzed. Mean digestibility of P bound to phytate was 94% with standard error of 1% (Table 6-6). Phosphorus bound to phytate was hydrolyzed *in vivo* when animals were fed a high concentration of phytate bound P.

TABLE 6-6. PERCENT OF PHYTATE BOUND P (PNY-P) HYDROLYZED IN FECES OF COWS IN TWO COMMERCIAL DAIRIES. HYDROLYSIS OF P BOUND TO PHYTATE DETERMINED BY INDIGESTIBLE MARKER TECHNIQUE USING ACID DETERGENT LIGNIN (ADL).

	Percent of feces as			
DAIRY[a]	PHY-P	ADL	P[b]	DIG COEF[c]
3	.17	13.5	1.19	79.79
3	.11	14.3	.99	87.65
3	.03	15.4	.46	96.87
3	.10	15.0	.89	89.30
3	.04	13.4	.80	95.21
3	.04	14.6	1.03	92.30
3	.05	14.4	1.05	94.43
3	.01	13.5	1.00	98.81
3	.03	16.2	.59	97.03
3	.03	13.6	1.05	96.46
3	.04	12.6	.71	94.90
3	.02	14.2	.69	97.74
3	.12	13.1	.72	85.30
4	.04	16.3	1.35	96.77
4	.03	16.4	.75	97.59
4	.04	16.5	.65	96.81
4	.04	17.6	.92	97.01
4	.04	17.8	1.13	97.04
4	.06	17.8	.83	95.56
4	.07	15.8	1.10	94.17

[a] Dairy: 3 = McArthur Farms (MF) Barn 3 (MF-3); 4 = MF 4.

[b] P = total P as % of fecal dry matter.

[c] Digestion coefficient (dig coef) determined as:
100 - (%ADL in feed / %ADL in feces * %phy-P in feces / %phy-P in feed) * 100,
where, ADL feed was 6.1 (4) or 5.0 (5);
phy p is phytate bound P,
and %phy-P feed is .38.

Summary

Ingrediants fed commonly to dairy cattle were analyzed for concentration of total P and P bound to phytate. Phosphorus bound to phytate represanted 55 to 80% of total P, except in dried distillers grain where P bound to phytata was 32% of total P. Disappearanca of P bound to phytata from ingrediants during in vitro fermentation for 6 h was least rapid for tha two high protein feeds evaluated (< 60%; soybean and cottonseed meai), and most rapid for grain by-products (> 70 %, dried distillers grain, hominy, rice bren, and wheat middlings)

Facal samples from cows consuming 17% of diet DM as P bound to phytate were analyzed. Greater than 99.9% of P bound to phytata was hydrolyzed when total collection of excreta or Cr was used as an indigestible marker to datarmine digestibility of phytata bound P. Whan lignin was used as an indigestible marker 94 to 98% of phytate bound P was hydrolyzed. Lignin was used as an indigestible marker to determine hydrolysis of phytate bound P in lactating cows fed a diet containing 38% phytata bound P. Minimum and maximum hydrolysis of P bound to phytata were 79.9 and 98.9%. Mean hydrolysis was 94.0 % with a standard arror of 1.0%.

These results indicated that P bound to phytate would be available from ingredients used to formulate diets for lactating cows. There was no evidenca from in vitro and in vivo results that discounting of P bound to phytate is necessary or valid when formulating diets for lactating cows.

CHAPTER 7
CONCLUSIONS

Phosphorus is an essential nutrient in diets of lactating dairy cows. This project addressed effects of modification of dietary P on excretion of P from dairy cows in commercial and research dairy herds. It further considered effects of concentration of dietary P on voluntary intake of feed and production of milk. Lastly, hydrolysis of P bound to phytate was examined.

In lactating dairy cows, P is excreted via feces, milk and urine. As intake of P was modified concentration of P in feces was modified accordingly. When concentration of P in concentrate portion of diet fed to cows on commercial dairies decreased, concentration of P in feces decreased. For samples obtained from one large commercial dairy (1,100 cows), as concentration of P in concentrate decreased from .75 (December) to .45% (January), concentration of P in feces decreased from 9158 to 5419 mg/kg of dried feces. Fecal samples were obtained from cows on two additional dairies where they were fed a diet containing .72% P. Concentration of P in feces obtained from cows during December and January decreased from 8677 to 7772 (MF-3) and from 9154 to 6976 mg/kg of dried feces (MF-4). When concentrations of P in feces were adjusted for month effects, mean concentration of P (mg of P/kg of DM) in feces collected from cows consuming a concentrate with .72% P was

9910 ± 150 (MF-4) and 9495 ± 150 (MF-3) on the dairy where cows also had access to free choice mineral containing 9.0% P.

Excretion of P in feces was most responsive to alterations in intake of P, whereas, excretion of P in milk was least responsive to alterations in intake of P during a short term (13 wk) experiment. Cows consuming 92 or 112 g of P/d retained P. Cows consuming 60 g of P/d consumed and excreted approximately equal quantities of P (cows were in balance). If zero net retention represented adequate intake of P, then 60 g of P/d was adequate for cows producing 22 to 25 kg of milk/d. As indicated previously, requirement for a mature 550 kg, nonpregnant cow, producing 20 kg of 4% FCM was 55.7 g of P/d. Concentration of P in a TMR would need to be .30% of DM if she consumed DM at 3.5% of her body weight. This amount of intake would provide adequate quantity of P (NRC, 1989). Recommended concentration of P in the diet for such a cow is .34% (NRC, 1989).

Quantity of P excreted in feces was reduced when intake of P was reduced from 92 to 60 g of P/d. Cows consuming 60 g of P/d excreted 41.9 g of P/d in feces after 9 wk on assigned diet. Some lower limit of excretion of P must exist regardless of dietary intake. Based on these results, it is suggested that the minimum quantity of P excreted in feces is near 40 g/d. Future research should address changes in excretion of P at intakes of P less than 80 g/d. A primary objective of such research would be to determine the quantity of intake of P needed for the animal to attain 0 retention of P. As formulation of diets for dairy cows becomes more refined (i.e., feeding specific limiting amino acids and B-vitamins), need for macro minerals likely will be determined on a g/d basis instead of the currently recommended concentrations

feeding known quantities of specific minerals will insure that daily intake is adequate for each animal. This will work most efficiently if cows are group fed according to production and age.

Evaluation of intake and excretion of P was in lactating cows. Further information is needed to determine excretion of P from other age groups of dairy cattle. Replacement heifers consume feed and produce excrete for approximately 2 yr before they enter the milking herd. Quantification of excretion of P from heifers at various stages of growth is necessary to determine if a reduction of excretion of P is the consequence of reduced intake.

For diets containing at least .33% P of diet DM, ratios of Ca:P in the diet between 1.1:1 and 2.9:1 did not effect voluntary intake of feed or quantity of milk produced. When concentration of P in diet DM was .33, .43, or .54%, neither voluntary intake of feed nor production of 3.5% or 4.0% fat-corrected milk differed ($p > .10$). Cows consuming a ration containing .33% P tended to produce more 3.5 and 4.0% FCM (24.0 and 22.2 kg/d) than cows consuming rations with .43 (23.3 and 21.6 kg/d) or .54% (23.2 and 21.5 kg/d) P. This trend was not significant ($p > .10$). As dietary concentration of Ca decreased from .97 to .60% of diet DM actual milk production increased from 21.9 to 22.7 ± .2 kg/d. Similarly production of 3.5 and 4.0% FCM increased from 23.0 to 24.0 ± .2 kg/d and from 21.3 to 22.2 ± .2 kg/d. Concentration of Ca in diet DM did not effect voluntary intake of feed. Dietary concentrations of P greater than currently recommended by NRC (1989) were not beneficial for milk production ($p > .10$). Dietary concentration of Ca at 150% of currently recommended concentrations (NRC, 1989) was detrimental to actual, 3.5 and 4.0% FCM production ($p < .05$).

Grain products fed to lactating dairy cows have a high proportion of P bound to phytate. Studies *in vitro* showed that P bound to phytate was liberated from the solids portion of the grain by 9 h of incubation. Subsequent hydrolysis of phytate bound P in solution by phytase was complete for most feedstuffs within 12 h of incubation. Cottonseed meal, high in protein, required more time (> 12 h) for phytate bound P to be hydrolyzed from the inositol ring. Results from *in vitro* incubations indicated that phytate bound P of feedstuffs commonly fed to dairy cows was hydrolyzed almost completely.

Analysis of fecal samples from dairy cows used in the total collection trial showed that less than 1% of dietary P bound to phytate was recovered in feces. Determination of hydrolysis of phytate bound P in feedstuffs fed to cows on commercial dairies utilized lignin as an indigestible marker. This technique revealed that greater than 95% of P bound to phytate was hydrolyzed.

Both the *in vitro* and *in vivo* results indicated that phytate bound P is hydrolyzed in dairy cattle. This occurs within the rumen. Based on these findings there is no evidence to indicate a need to expect reduced availability of the P bound to phytate in most feedstuffs when formulating diets for dairy cattle. Therefore, when formulating diets for dairy cows one should consider all P in feedstuffs available to the animal. Although both *in vitro* and *in vivo* results were positive, additional research is needed. It will be necessary to determine time required for complete or maximal hydrolysis of phytate bound P and also to determine the biological availability of P hydrolyzed from phytate to lactating cows. This would determine if there is any need to discount availability of P bound to phytate in specific feedstuffs, specifically

those of small sized particles. Future research should address interactions between high protein feeds and cereal grains and the ability of phytase to hydrolyze phytate bound P. Experiments *in situ* will be required to enhance understanding of interactions of level of protein and phytate bound P availability for lactating dairy cows. Additional *in vitro* research should evaluate source of rumen fluid. When dietary components are altered microbial ecology of the rumen is altered. Microorganisms in rumen fluid obtained from animals consuming all forage diets may hydrolyze phytate bound P less well than microorganisms in rumen fluid obtained from animals consuming a high concentrate diet.

Abbreviations used in Appendices A through D.

Term	Definition
P	Concentration of P in feces (ppm/g DM)
P out	Estimated excretion of P (g/d)
μ	Population mean
DIM	Days in milk
Age	Age of cow (months since birth)
Milk	Milk produced at most recent sample (kg/d)
Dairy	Dairy farm
Month	Month of year
Pdiet	Concentration of P in concentrate portion of diet
Breed	Holstein or Jersey
Parity	Lactation number
DF	Degrees of freedom associated with source
SS	Sum of squares
Type III SS	Sequential SS associated with source
F	Mean square source/mean square error
PR	Level of significance
R^2	Degree to which model explains variation in dependant variable. Calculated as Model SS/Total SS
CV	Coefficient of variation
Mean	Least squares mean for dependent variable adjusted for components in model
SE	Standard error of the mean

APPENDIX A

LEAST SQUARES ANALYSIS OF VARIANCE OF CONCENTRATION OF PHOSPHORUS IN FECES BY DAIRY AND MONTH FOR DATA DESCRIBED IN CHAPTER 3

Analyses used 301 (with DIM, with PARITY), 302 (with DIM and without PARITY), or 688 (no DIM or PARITY) observations. These observations were from McArthur farms (MF) or Dry Lake Dairies (DLD) and had values for DIM, MILK, AGE, PARITY, AND BHEED.

TABLE A-1. LEAST SQUARES ANALYSIS OF VARIANCE OF CONCENTRATION OF PHOSPHORUS IN FECES (MG OF PHOSPHORUS/KG OF DM).

Model : P = μ + DIM + DIM*DIM + MILK + AGE + DAIHY + MONTH
+ DAIRY*MONTH + PARITY + BHEED + DAIRY*BHEED
+ MONTH*BHEED + DAIHY*MONTH*BHEED.

Least Squares Analysis of Variance

Source	DF	Type III SS	F	PR
DIM	1	363990.94	012	7320
DIM*DIM	1	8236443.75	2.66	1042
MILK	1	2835770.70	.92	.3396
AGE	1	193.39	.00	9336
DAIRY (D)	3	46340573.38	4.99	.0022
MONTH (M)	6	241093415.12	12.97	.0001
D*M	9	222350468.44	7.97	.0001
PARITY	3	10426822.25	1.12	.3407
BREED (B)	1	423262.65	.14	.7120
D*B	2	438986.02	.71	.4933
M*B	6	1145327.47	.62	.7173
D*M*B	6	9223380.83	.60	.7035
ERROR	261	808572615.78		

R^2 = .60
CV = 21.20
P Mean = 8301 ppm

TABLE A-2. LEAST SQUARES ANALYSIS OF VARIANCE OF CONCENTRATION OF PHOSPHORUS IN FECES (MG OF PHOSPHORUS/KG OF DM).

Model 2 : $P = \mu + DIM + DIM*DIM + MILK + AGE + DAIRY + MONTH + DAIRY*MONTH + PARITY$

Least Squares Analysis of Variance

SOURCE	DF	TYPE III SS	F	PR
DIM	1	290904.64	.10	.7320
DIM*DIM	1	10191691.53	3.36	.0679
MILK	1	2435770.70	1.36	.2441
AGE	1	186960.80	.06	.8042
DAIRY (D)	3	83977127.92	9.22	.0001
MONTH (M)	6	376409849.87	20.67	.0001
D*M	9	252839605.23	9.26	.0001
PARITY	3	8049778.90	.88	.4497
ERROR	275	834478015.13		

R^2 = .59
CV = 20.99
P Mean = 8302 ppm

TABLE A-3. LEAST SQUARES ANALYSIS OF VARIANCE OF CONCENTRATION OF PHOSPHORUS IN FECES (MG OF PHOSPHORUS/KG OF DM).

Model 3 : $P = \mu + DIM + DIM*DIM + MILK + DAIRY + MONTH + DAIRY*MONTH + PARITY$

Least Squares Analysis of Variance

SOURCE	DF	TYPE III SS	F	PR
DIM	1	363990.94	.07	.7803
DIM*DIM	1	11923080.89	3.98	.0479
MILK	1	3668079.04	1.25	.2704
DAIRY (D)	3	84692383.07	9.61	.0001
MONTH (M)	6	385165177.09	9.56	.0001
D*M	9	256055500.06	20.98	.0001
PARITY	3	7803471.11	.85	.4688
ERROR	275	842527794.04		

R^2 = .59
CV = 20.89
P Mean = 8312 ppm

TABLE A-4. LEAST SQUARES ANALYSIS OF VARIANCE AND LEAST SQUARES MEANS OF CONCENTRATION OF PHOSPHORUS IN FECES (MG OF PHOSPHORUS/KG OF DM).

Model 4 : P = μ + DIM + DIM*DIM + MILK + DAIRY + MONTH + DAIRY*MONTH

Least Squares Analysis of Variance

SOURCE	DF	TYPE III SS	F	PR
DIM	1	241107.01	.08	.7774
DIM*DIM	1	11910804.32	3.96	.0477
MILK	1	3890696.73	1.28	.2584
DAIRY (D)	3	89920222.95	9.96	.0001
MONTH (M)	6	386055595.58	21.37	.0001
D*M	9	256053031.91	9.45	.0001
ERROR	280	842853682.13		

Least Squares Means

Least squares mean concentration of P in feces by dairy and month.

	Month						
Dairy	Nov	Dec	Jan	Feb	Mar	July	Aug
OLD 2		7298	5901				
MF-1	11092	9198	5364	5835	4918		
MF-3	9300	8497	8171	9596		10443	9838
MF-4	9229	8502	6927	8806	8067	10588	

SE range 404 to 506, except OLD-2 Jan SE = 712.

R^2 = .59
CV = 20.87
P Mean = 8312 ppm

TABLE A-6. LEAST SQUARES ANALYSIS OF VARIANCE AND LEAST SQUARES MEANS OF CONCENTRATION OF PHOSPHORUS IN FECES (MG OF PHOSPHORUS/KG OF DM).

Model 5 : $P = \mu + DIM + DIM*DIM + DAIRY + MONTH + DAIRY*MONTH$

Least Squares Analysis of Variance

SOURCE	DF	TYPE III SS	F	PR
DIM	1	1017401.99	.34	.5617
DIM*DIM	1	10445417.58	3.47	.0637
DAIRY (D)	3	88563640.66	9.80	.0001
MONTH (M)	6	405518906.25	22.43	.0001
D*M	9	26614275.42	9.81	.0001
ERROR	281	846714378.86		

Least Squares Means

Least squares mean concentration of P in feces by dairy and month.

	Month						
Dairy	Nov	Dec	Jan	Feb	Mar	July	Aug
OLO-2		7354	5929				
MF-1	11152	9152	5400	5784	4848		
MF-3	9289	8539	8200	9671		10545	9939
MF-4	9165	8460	6881	8740	7948	10516	

R^2 = .59
CV = 20.88
P Mean = 8312 ppm

TABLE A-6. LEAST SQUARES ANALYSIS OF VARIANCE AND LEAST SQUARES MEANS OF CONCENTRATION OF PHOSPHORUS IN FECES (MG OF PHOSPHORUS/KG OF DM).

Model G : $P = \mu +$ DAIRY + MONTH + DAIRY*MONTH

Least Squares Analysis of Variance

SOURCE	DF	TYPE III SS	F	PR
DAIRY (D)	3	121439191.85	12.10	.0001
MONTH (M)	6	423508557.41	21.10	.0001
D*M	9	326059204.39	10.83	.0001
ERROR	283	946646302.09		

Least Squares Means

Least squares mean concentration of P in feces by dairy and month.

	Month						
Dairy	Nov	Dec	Jan	Feb	Mar	July	Aug
OLD-2		6881	5576				
MF-1	11320	8976	5371	5490	4816		
MF-3	9041	8576	7894	9953		10977	10371
MF-4	8954	8788	6926	8826	8167	10492	

R^2 = .54
CV = 22.00
P Mean = 8312 ppm

TABLE A-7. LEAST SQUARES ANALYSIS OF VARIANCE AND LEAST SQUARES MEANS OF CONCENTRATION OF PHOSPHORUS IN FECES (MG OF PHOSPHORUS/KG OF DM).

Model 7 : P = μ + DAIRY + MONTH + DAIRY*MONTH

Least Squares Analysis of Variance

SOURCE	DF	TYPE III SS	F	PR
DAIRY (D)	4	684458619.38	67.34	.0001
MONTH (M)	6	894805520.52	34.61	.0001
D*M	24	783802363.08	10.14	.0001
ERROR	549	2106793905.67		

Least Squares Means

Least squares mean concentration of P in feces by dairy and month.

	Month						
Dairy	Nov	Dec	Jan	Feb	Mar	July	Aug
OLO-1	7105	5625	5958	6204	6959	9710	9662
OLO-2	5592	6882	6047	5691	5859	6144	8192
MF-1	11347	9158	5419	5513	5021	9469	7072
MF-3	9267	8677	7772	10100	9428	10857	10366
MF-4	8781	9154	6976	8925	8215	11039	9275

R^2 = .54
CV = 22.00
P Mean = 8312 ppm

APPENDIX B
LEAST SQUARES ANALYSIS OF VARIANCE OF
CONCENTRATION OF PHOSPHORUS IN FECES
BY CONCENTARTION OF PHOSPHORUS IM CONCENTRATE
FOR DATA DESCRIBED IM CHAPTER 3

Analyses used 301 (with DIM, with PARITY), 302 (with DIM and without PARITY), or 698 (no DIM or PARITY) observations. These observations were from McArthur farms (MF) or Dry Lake Dairies (DLD) and had values for DIM, MILK, AGE, PARITY, ARD BREED.

TABLE B-1. LEAST SQUARES ARALYSIS OF VARIARCE OF CONCEMTRATION OF PHOSPHORUS IN FECES (MG OF PHOSPHORUS/KG OF DM).

Model 1 : P = μ + DIM + DIM*DIM + AGE + MILK + PDIET
+ PDIET*PDIET + BREED + PARITY + BREED*PARITY
+ BREED*PDIET + BREED*PDIET*PDIET.

Least Squares Analysis of Variance

SOURCE	DF	TYPE III SS	F	PR
DIM	1	4657772.50	1.20	.2742
DIM*DIM	1	35470357.27	9.15	.0027
AGE	1	24194.10	.01	.9371
MILK	1	29188863.08	7.53	.0064
PDIET	1	14232604.60	3.67	.0563
PDIET*PDIET	1	8652952.85	2.23	.1362
BREED (B)	1	38770.97	.01	.9204
PARITY (PAR)	3	9017705.07	.78	.5084
B*PAR	3	1900963.92	.16	.9209
B*PDIET	1	34601.95	.01	.9248
B*PDIET*PDIET	1	33973.70	.01	.9255
ERROR	285	1104489387.86		

R^2 = .46
CV = 23.72
P Mean = 8301 ppm

TABLE 8-2. LEAST SQUARES ANALYSIS OF VARIANCE OF CONCENTRATION OF PHOSPHORUS IN FECES (MG OF PHOSPHORUS/KG OF DM).

Model 2 : $P = \mu + DIM + DIM*DIM + MILK + PDIET + PDIET*PDIET + BREED + PARITY + BREED*PARITY + BREED*PDIET + BREED*PDIET*PDIET$

Least Squares Analysis of Variance

SOURCE	DF	TYPE III SS	F	PR
DIM	1	4441959.00	1.15	.2847
DIM*DIM	1	35319235.36	9.13	.0027
MILK	1	29278192.32	7.57	.0063
PDIET	1	11590432.52	3.00	.0845
PDIET*PDIET	1	6509014.90	1.68	.1955
BREED (B)	1	31314.99	.01	.9284
PARITY (PAR)	3	8662405.87	.75	.5250
B*PAR	3	2618717.18	.23	.8785
B*PDIET	1	39277.57	.01	.9198
B*PDIET*PDIET	1	43985.43	.01	.9151
ERROR	287	1109757350.33		

R^2 = .46
CV = 23.66
P Mean = 8312 ppm

TABLE 8-3. LEAST SQUARES ANALYSIS OF VARIANCE OF CONCENTRATION OF PHOSPHORUS IN FECES (MG OF PHOSPHORUS/KG OF DM)

Model 3 : $P = \mu + DIM + DIM*DIM + MILK + PDIET + PDIET*PDIET + PARITY$

Least Squares Analysis of Variance

SOURCE	DF	TYPE III SS	F	PR
DIM	1	4595274.53	1.21	.2722
DIM*DIM	1	36804143.19	9.69	.0020
MILK	1	38966156.45	10.26	.0015
PDIET	1	13664557.15	3.60	.0588
PDIET*PDIET	1	7892715.28	2.08	.1505
PARITY	3	10403391.35	.91	.4346
ERROR	293	1112597284.56		

R^2 = .46
CV = 23.44
P Mean = 8312 ppm

TABLE 8-4. LEAST SQUARES ANALYSIS OF VARIANCE OF CONCENTRATION OF PHOSPHORUS IN FECES (MG OF PHOSPHORUS/KG OF DM).

Model 4: $P = \mu + DIM + DIM*DIM + MILK + PDIET + PDIET*PDIET$

Least Squares Analysis of Variance

SOURCE	DF	TYPE III SS	F	PR
DIM	1	7963407.25	2.10	.1495
DIM*DIM	1	44244654.70	11.66	.0007
MILK	1	50592092.57	13.33	.0003
PDIET	1	19762241.07	5.21	.0232
PDIET*PDIET	1	12464655.67	3.29	.0709
ERROR	296	1123006675.91		

R^2 = .46
CV = 23.72
P Mean = 8301 ppm

TABLE 8-5. LEAST SQUARES ANALYSIS OF VARIANCE OF CONCENTRATION OF PHOSPHORUS IN FECES (MG OF PHOSPHORUS/KG OF DM).

Model 5: $P = \mu + DIM + DIM*DIM + MILK + PDIET$

Least Squares Analysis of Variance

SOURCE	DF	TYPE III SS	F	PR
DIM	1	6522572.57	1.71	.1925
DIM*DIM	1	42991982.32	11.25	.0009
MILK	1	60653308.13	15.86	.0001
PDIET	1	581034282.20	151.98	.0001
ERROR	297	1136471331.58		

R^2 = .45
CV = 23.52
P Mean = 8312 ppm

TABLE 8-6. LEAST SQUARES ANALYSIS OF VARIANCE OF CONCENTRATION OF PHOSPHORUS IN FECES (MG OF PHOSPHORUS/KG OF DM).

Model 6. $P = \mu + POIET$

POIET was retained to compare results from the reduced data set (302 observations) and the complete data set (688 observations).

Least Squares Analysis of Variance

SOURCE	DF	TYPE III SS	F	PR
POIET	1	697679553.97	268.26	.0001
ERROR	300	1359466943.35		

R^2 = .34
CV = 25.61
P Mean = 7920 ppm

TABLE 8-7. LEAST SQUARES ANALYSIS OF VARIANCE OF CONCENTRATION OF PHOSPHORUS IN FECES (MG OF PHOSPHORUS/KG OF DM).

Model 7 $P = \mu + POIET$

Least Squares Analysis of Variance

SOURCE	DF	TYPE III SS	F	PR
POIET	1	1261642991.00	268.25	0001
ERROR	686	3226343278.59		

R^2 = .28
CV = 27.38
P Mean = 7920 ppm

APPENDIX C
LEAST SQUARES ANALYSIS OF VARIANCE OF CONCENTRATION
OF PHOSPHORUS IN FECES
BY ESTIMATED DAILY INTAKE OF PHOSPHORUS (G/D)
FOR DATA DESCRIBED IN CHAPTER 3

Analyses used 276 (with PARITY) or 277 (without PARITY) observations. These observations were from McArthur farms and had values for DIM, MILK, AGE, PARITY, AND BREED.

TABLE C.1 LEAST SQUARES ANALYSIS OF VARIANCE OF CONCENTRATION OF PHOSPHORUS IN FECES (MG OF PHOSPHORUS/KG OF DM).

Model 1 $P = \mu + DIM + DIM*DIM + AGE + MILK + PIN + PIN*PIN + BRFFD + PARITY + BREED*PARITY + PIN*BREED + PIN*PIN*BRFED$

Least Squares Analysis of Variance

SOURCE	DF	TYPE III SS	F	PR
DIM	1	330761.04	.08	.7723
DIM*DIM	1	22006929.58	5.58	.0189
AGE	1	68923.61	.02	.8849
MILK	1	15687753.07	3.98	.0471
PIN	1	24012959.22	60.94	.0001
PIN*PIN	1	20830180.95	52.86	.0001
BREED (B)	1	3968867.30	1.01	.3165
PARITY (PAR)	3	4571893.67	.39	.7627
B*PAR	3	17437090.89	1.47	.2217
B*PIN	1	5698913.59	1.45	.2302
B*PIN*PIN	1	7207995.56	1.83	.1774
ERROR	260	1024554038.61		

R^2 = .45
CV = 23.47
P Mean = 8458 ppm

TABLE C-2 LEAST SQUARES ANALYSIS OF VARIANCE OF CONCENTRATION OF PHOSPHORUS IN FECES (MG OF PHOSPHORUS/KG OF DM).

Model 2 : $P = \mu + DIM + DIM*DIM + MILK + PIN + PIN*PIN + BNEED + PARITY + BNEED*PARITY + PIN*BREED + PIN*PIN*BNEED$

Least Squares Analysis of Variance

SOURCE	DF	TYPE III SS	F	PR
DIM	1	415247.34	.31	.7453
DIM*DIM	1	21443918.06	5.46	.0202
MILK	1	15927159.15	4.06	.0450
PIN	1	244688895.48	62.32	.0001
PIN*PIN	1	212363086.29	54.09	.0001
BNEED (B)	1	4458221.16	1.15	.2854
PARITY (PAR)	3	4397960.52	.37	.7723
B*PAR	3	16821592.36	1.43	.2349
B*PIN	1	6351155.06	1.62	.2045
B*PIN*PIN	1	7922004.22	2.02	.1567
ERROR	262	1028559449.87		

R^2 = .45
CV = 23.40
P Mean = 8469 ppm

TABLE C-3 LEAST SQUARES ANALYSIS OF VARIANCE OF CONCENTRATION OF PHOSPHORUS IN FECES (MG OF PHOSPHORUS/KG OF DM)

Model 3 : $P = \mu + DIM + DIM*DIM + MILK + PIN + PIN*PIN + BNEED + PIN*BNEED + PIN*PIN*BNEED$

Least Squares Analysis of Variance

SOURCE	DF	TYPE III SS	F	PR
DIM	1	81.51	.00	.9964
DIM*DIM	1	26805230.72	6.83	.0095
MILK	1	13928040.51	3.55	.0606
PIN	1	244466433.28	62.32	.0001
PIN*PIN	1	214153953.75	54.59	.0001
BNEED (B)	1	4207937.24	1.07	.3013
B*PIN	1	6194792.18	1.58	.2100
B*PIN*PIN	1	8036146.52	2.05	.1535
ERROR	268	1051278331.73		

R^2 = .43
CV = 23.39
P Mean = 8469 ppm

TABLE C-4. LEAST SQUARES ANALYSIS OF VARIANCE OF CONCENTRATION OF PHOSPHORUS IN FECES (MG OF PHOSPHORUS/KG OF DM).

Model 4: $P = \mu + DIM + DIM*DIM + MILK + PIN + PIN*PIN$

Least Squares Analysis of Variance

SOURCE	DF	TYPE III SS	F	PR
DIM	1	607389.00	.15	.6960
DIM*DIM	1	30243999.65	7.62	.0062
MILK	1	35934307.58	9.05	.0029
PIN	1	333149649.63	83.89	.0001
PIN*PIN	1	291315223.34	73.35	.0001
ERROR	271	1076231877.31		

R^2 = .42
CV = 23.53
P Mean = 8469 ppm

TABLE C-5. LEAST SQUARES ANALYSIS OF VARIANCE AND PARAMETER ESTIMATES OF CONCENTRATION OF PHOSPHORUS IN FECES (MG OF PHOSPHORUS/KG OF DM).

Model 5: $P = \mu + PIN + PIN*PIN$

Least Squares Analysis of Variance

SOURCE	DF	TYPE III SS	F	PR
PIN	1	418242397.75	87.47	.0001
PIN*PIN	1	371519405.71	77.70	.0001
ERROR	274	1310198859.35		

Parameter Estimates

Parameter	Estimate	SE
Intercept	-34553.09	4383.85
PIN	764.16	81.71
PIN*PIN	-3.31	.38

R^2 = .29
CV = 25.82
P Mean = 8469 ppm

TABLE C-6. LFAST SQUARES ANALYSIS OF VARIANCE OF CONCENTRATION OF PHOSPHORUS IN FECES (MG OF PHOSPHORUS/KG OF DM).

Model 6 : $P = \mu + PIN + PIN*PIN$

Least Squares Analysis of Variance

SOURCE	DF	TYPE III SS	F	PR
PIN	1	276012569.84	51.52	.0001
PIN*PIN	1	240955001.42	44.98	.0001
ERROR	407	2180374202.26		

R^2 = .15
CV = 26.73
P Mean = 8659 ppm

APPENDIX D
LEAST SQUARES ANALYSIS OF VARIANCE OF DAILY EXCRETION OF PHOSPHORUS
FOR DATA DESCRIBED IN CHAPTER 3

TABLE D.1 LEAST SQUARES ANALYSIS OF VARIANCE AND LEAST SQUARES MEANS OF ESTIMATED EXCRETION OF PHOSPHORUS IN FECES (G/D).

Model 1 P out = μ + DAIRY + MONTH + DAIRY*MONTH

Least Squares Analysis of Variance

SOURCE	DF	TYPE III SS	F	PR
DAIRY	2	665.44	1.46	.2341
MONTH	3	12352.62	18.09	.0001
DAIRY*MONTH	6	12559.74	9.20	.0001

Least Squares Means

Least squares mean estimated excretion of P in feces by dairy and month.

	Month				
Dairy	Nov	Dec	Jan	Feb	SE
MF-1	73.82	66.65	35.01	45.47	3.37
MF-3	59.35	60.50	51.41	70.14	3.37
MF-4	62.91	51.74	45.29	69.23	3.56

R^2 = .35
CV = 25.57
P out Mean = 58.08 g/d

TABLE D-2. LEAST SQUARES ANALYSIS OF VARIANCE OF ESTIMATED EXCRETION OF PHOSPHORUS IN FECES (G/D).

Model 2 P out = μ + DIM + DIM*DIM + AGE + MILK + PDIET
+ PDIET*PDIET + BREED + PARITY
+ BREED*PARITY + BREED*PDIET
+ BREED*PDIET*PDIET

Least Squares Analysis of Variance

SOURCE	DF	TYPE III SS	F	PR
DIM	1	191.76	.83	.3625
DIM*DIM	1	212.59	.92	.3377
AGE	1	53.41	.23	.6305
MILK	1	.06	.00	.9868
PDIET	1	1221.73	6.31	.0224
PDIET*PDIET	1	1381.17	6.00	.0153
BREED (B)	1	155.47	.68	.4121
PARITY (PAR)	3	880.81	1.28	.2840
B*PAR	3	260.69	.38	.7691
B*PDIET	1	139.15	.60	.4377
B*PDIET*PDIET	1	130.32	.57	.4526
ERROR	175	40251.57		

R^2 = .31
CV = 26.57
P out Mean = 57.07 g/d

TABLE D-3 LEAST SQUARES ANALYSIS OF VARIANCE OF ESTIMATED EXCRETION OF PHOSPHORUS IN FECES (G/D).

Model 3 : P out = μ + DIM + DIM*DIM + AGE + MILK + PIN
+ PIN*PIN + BREED + PARITY + BREED*PARITY
+ BREED*PIN + BREED*PIN*PIN.

Least Squares Analysis of Variance

SOURCE	DF	TYPE III SS	F	PR
DIM	1	170.57	.74	.3919
DIM*DIM	1	231.08	1.00	.3192
AGE	1	32.60	.15	.6955
MILK	1	94.92	.41	.5229
PIN	1	4055.94	17.62	.0001
PIN*PIN	1	3636.35	15.70	.0001
BREED (B)	1	93.39	.40	.5262
PARITY (PAR)	3	765.91	1.11	.3476
B*PAR	3	297.93	.43	.7326
B*PIN	1	74.11	.32	.5723
B*PIN*PIN	1	57.44	.25	.6191
ERROR	175	40524.34		

R^2 = .31
CV = 26.66
P out Mean = 58.07 g/d

List of abbreviations used in Appendices E through H

Term	Definition
DF	degrees of freedom
Type III SS	sequential sums of squares
F	mean square source/mean square error
PR	level of significance
SE	standard error of the mean
D	diet
Period A	days 2, 3, and 4 of balance
Period B	days 5, 6, and 7 of balance
L	30% P in diet DM
M	41% P in diet DM
H	56% P in diet DM
Balance 1	cows consumed M diet
Balances 2, 3, and 4	cows consumed assigned diets for 3, 6, and 9 wk
Coll	total collection technique
Cr	Chromium
ADL	acid detergent lignin

Cow(D) used as error term for tests of significance for D (Appendices E through G)

APPENDIX E
LEAST SQUARES ANALYSIS OF VARIANCE OF ROUTE OF EXCRETION OF PHOSPHORUS FOR DATA DESCRIBED IN CHAPTER 4

TABLE E-1. LEAST SQUARES ANALYSIS OF VARIANCE AND MEANS OF EXCRETION OF PHOSPHORUS IN FECES (G/D).

Least Squares Analysis of Variance

	BALANCE 1				BALANCES 2, 3, & 4			
Source	DF	Type III SS	F	Pr > F	DF	Type III SS	F	Pr > F
0	2	372.35	1.42	.2903	2	11840.46	76.30	.0001
Cow(D)	9	1177.21	6.00	.0068	9	698.34	1.84	.0901
Period	1	135.22	6.20	.0344	5	1457.10	6.92	.0001
D*Period	2	55.55	1.27	.3257	10	304.33	.72	.6962
Error	8	349.56			40	1050.7		

Contrast	DF	Type III SS	F	Pr > F	DF	Type III SS	F	Pr > F
L vs M & H	1	202.89	1.55	.2444	1	5813.92	74.93	.0001
M vs H	1	169.46	1.30	.2844	1	4208.14	54.23	.0001
1A vs 1B	1	135.22	6.20	.0344				
2A vs 2B					1	7.70	.18	.6711
3A vs 3B					1	59.48	1.41	.2416
4A vs 4B					1	3.87	.11	.7393

Least Squares Means

Diet	Mean	SE	Mean	SE
L	53.74	4.04	35.66	1.86
M	44.32	4.04	45.36	2.32
H	50.83	4.04	66.93	1.80

Least Squares Means

	Period									
Diet	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B
L	54.86	52.61	2.31	30.82	33.95	36.07	30.09	42.74	40.40	3.24
M	45.79	42.85	2.31	40.72	42.30	46.68	44.27	48.18	50.00	3.97
H	50.91	46.81	2.31	59.67	58.35	67.33	63.4	71.01	71.0	3.24

TABLE E-2. LEAST SQUARES ANALYSIS OF VARIANCE AND MEANS OF EXCRETION OF PHOSPHORUS IN MILK (G/D).

Least Squares Analysis of Variance

	BALANCE 1				BALANCES 2, 3, & 4			
Source	DF	Type III SS	F	Pr > F	DF	Type III SS	F	Pr > F
D	2	80.70	.99	.4066	2	114.95	.68	.5300
Cow(D)	9	366.73	12.69	.0004	9	758.58	23.78	.0001
Period	1	.79	.25	.6316	5	150.50	8.48	.0001
D*Period	2	15.89	2.47	.1393	10	80.46	2.25	.0185
Error	9	28.89			45	161.91		

Contrast	DF	Type III SS	F	Pr > F	DF	Type III SS	F	Pr > F
L vs M & H	1	50.06	1.47	.2556	1	83.79	.99	.3448
M vs H	1	20.64	.51	.4947	1	16.77	.20	.6661
1A vs 1B	1	.79	.26	.6316				
2A vs 2B					1	.65	.18	.6719
3A vs 3B					1	8.02	2.26	.1403
4A vs 4B					1	4.08	1.91	.2188

Least Squares Means

Diet	Mean	SE	Mean	SE
L	19.68	2.56	18.13	1.94
M	21.90	2.56	19.90	2.42
H	24.17	2.56	21.27	1.87

Least Squares Means

	Period								
Diet	1	2	SE	3	4	5	6	7	SE
L	20.63	18.73	.90	18.45	18.42	17.02	19.29	19.23	16.31	.94
M	21.34	22.47	.90	23.91	23.74	18.06	18.36	18.24	17.13	1.15
H	23.43	25.11	.90	25.68	23.74	19.41	20.06	19.79	21.12	.81

TABLE E-3. LEAST SQUARES ANALYSIS OF VARIANCE AND MEANS OF EXCRETION OF PHOSPHORUS IN URINE (G/D).

Least Squares Analysis of Variance

		BALANCE 1				BALANCES 2, 3, & 4			
Source	DF	Type III SS	F	Pr > F	DF	Type III SS	F	Pr > F
D	2	2.56	2.42	.1443	2	151.04	4.50	.0442
Cow(D)	9	5.35	.76	.7127	9	151.03	15.69	.0001
Period	1	.14	.19	.7384	5	7.67	1.43	.2334
D*Period	2	3.93	2.58	.4028	10	18.51	1.73	.1082
Error		.76			30	41.61		

Contrast	DF	Type III SS	F	Pr > F	DF	Type III SS	F	Pr > F
L vs M & H	1	2.31	3.58	.0803	1	55.88	3.33	.1013
M vs H	1	1.01	1.71	.2238	1	48.54	2.89	.1232
1A vs 1B	1	.14	.19	.7384				
2A vs 2B					1	2.69	2.57	.1168
3A vs 3B					1	1.64	1.57	.2171
4A vs 4B					1	2.07	1.98	.1675

Least Squares Means

Diet	Mean	SE	Mean	SE
L	.05	.55	.70	.86
M	1.65	.55	1.49	1.37
H	.78	.39	4.21	.84

Least Squares Means

	Period									
Diet	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B
L	.80	.71	1.15	.70	1.05	.85	.56	.67	.58	.51
M	2.85	.45	1.15	1.36	1.53	1.54	1.30	1.61	1.60	.67
H	.78	.45	.35	2.58	4.18	1.80	1.58	1.28	3.08	.88

TABLE E-4. LEAST SQUARES ANALYSIS OF VARIANCE AND MEANS OF TOTAL EXCRETION OF PHOSPHORUS (G/D).

Least Squares Analysis of Variance

	BALANCE 1				BALANCES 2, 3, & 4			
Source	DF	Type III SS	F	PR > F	DF	Type III SS	F	PR > F
D	2	46.35	.27	.7678	2	17318.25	122.98	.0001
Cow(D)	9	766.24	2.54	.4543	9	633.72	1.47	.1919
Period	1	23.61	.70	.5657	5	982.14	4.11	.0043
D*Period	2	22.39	.33	.7745	10	409.19	.86	.5796
Error	1	33.81			60	855.1		

Contrast	DF	Type III SS	F	PR > F	DF	Type III SS	F	PR > F
L vs M & H	1	1.09	.01	.9123	1	7179.88	101.97	.0001
M vs H	1	41.51	.49	.5027	1	4840.93	68.75	.0001
1A vs 1B	1	23.61	.70	.5557				
2A vs 2B					1	27.22	.57	.4548
3A vs 3B					1	12.94	.27	.6056
4A vs 4B					1	36.33	.77	.3848

Least Squares Means

Diet	Mean	SE	Mean	SE
L	71.24	6.52	54.51	1.77
M	69.32	6.52	65.27	2.80
H	74.90	4.61	92.46	1.71

Lease Squares Means

	Period										
Diet	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B	SE
L	70.44	72.05	4.23	49.97	53.43	53.73	49.94	62.69	57.29	3.45	
M	72.87	65.77	4.23	63.03	57.56	64.80	64.45	66.54	67.24	4.45	
H	71.69	77.21	3.66	85.83	96.19	90.71	92.07	104.3	96.85	3.45	

TABLE E-5. LEAST SQUARES ANALYSIS OF VARIANCE AND MEANS OF MILK PRODUCTION (KG/D).

Least Squares Analysis of Variance

	BALANCE 1				BALANCES 2, 3, & 4			
Source	df	Type III SS	F	Pr > F	df	Type III SS	F	Pr > F
D	2	76.99	94	4260	2	107.93	73	5001
Cow (D)	9	268.22	19.53	.0001	9	666.83	37.58	.0001
Period	1	.14	.07	.7988	5	46.65	4.73	.0017
D*Period	2	3.14	.75	.5002	10	60.79	3.08	.0054
Error	8	18.06			40	78.86		

Contrast	df	Type III SS	F	Pr > F	df	Type III SS	F	Pr > F
L vs M & H	1	73.99	1.81	.2116	1	105.15	1.42	.2640
M vs H	1	2.90	.07	.7961	1	.00	.00	.9549
1A vs 1B	1	.14	.07	.7988				
2A vs 2B					1	2.24	1.14	.2924
3A vs 3B					1	1.62	.82	.3700
4A vs 4B					1	1.54	1.01	.3178

Least Squares Means

Diet	Mean	SE	Mean	SE
L	19.93	2.26	19.03	1.81
M	23.23	2.26	21.77	2.27
H	24.08	2.26	21.79	1.76

Least Squares Means

	Period									
Diet	1A	1B	2A	2B	3A	3B	4A	4B	SE	
L	20.34	19.51	72	18.30	18.61	19.58	20.04	20.01	18.33	.70
M	23.03	23.43	72	24.25	24.46	20.27	21.38	20.43	19.86	.86
H	23.83	24.32	.71	23.61	24.70	21.13	21.39	21.22	19.72	.86

APPENDIX F
LEAST SQUARES ANALYSIS OF VARIANCE OF ROUTE OF EXCRETION OF PHOSPHORUS FOR DATA DESCRIBED IN CHAPTER 4

TABLE F-1. LEAST SQUARES ANALYSIS OF VARIANCE AND MEANS OF EXCRETION OF PHOSPHORUS IN FECES (G/D).

Least Squares Analysis of Variance

		BALANCE 1			BALANCES 2, 3, & 4		
	DF	Type III SS	F	Pr	DF	Type III SS	F	Pr
D	2	372.35	1.42	2903	2	11781.30	75.71	0001
Cow(D)	9	1177.21	4.06	0136	9	691.13	1.99	0608
Balance					2	1356.36	17.57	0001
D*Balance					4	198.98	1.29	2874
Error	12	367.[illegible]			44	1701.[illegible]		

Contrast	DF	Contrast SS	F	Pr	DF	Type III SS	F	Pr
L vs M & H	1	202.89	1.55	.2444	1	5756.97	74.97	0001
M vs H	1	169.43	1.30	2948	1	[illegible]	24.[illegible]	[illegible]

Least Squares Means

Diet	Mean	SE	Mean	SE
L	53.74	4.04	35.84	1.84
M	44.32	4.04	45.36	2.31
H	50.83	4.04	66.98	1.79

Least Squares Means

	Balance					
Diet	1	SE	2	3	4	SE
L	53.74	4.04	32.39	33.57	41.57	2.20
M	44.32	4.04	41.51	45.48	49.09	2.53
H	50.83	4.04	59.01	66.40	75.53	2.20

TABLE F-2. LEAST SQUARES ANALYSIS OF VARIANCE AND MEANS OF EXCRETION OF PHOSPHORUS IN MILK (G/D)

Least Squares Analysis of Variance

	BALANCE 1				BALANCES 2, 3, & 4			
	F				F			
D	2	80.70	.99	.4086	2	120.41	.72	.5119
Cow(D)	9	366.73	10.73	.0002	9	750.40	22.89	.0001
Balance					2	138.02	18.95	.0001
D*Balance					4	65.60	4.50	.0035
[illegible]	[illegible]	4.59			[illegible]	47[illegible]		

	F				F			
L Vs M & H	1	60.06	1.47	.2556	1	88.72	1.06	.3292
[illegible]	1	20.64	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	.60[illegible]

Least Squares Means

Diet	Mean	SE	Mean	SE
L	19.68	2.26	18.01	1.91
M	21.90	2.26	19.30	2.41
H	24.17	2.26	21.27	1.86

Least Squares Means

			Balance			
Diet	1	SE	2	3	4	SE
L	19.68	2.26	18.44	17.96	17.79	.68
M	21.90	2.26	23.83	18.21	17.68	.87
H	24.17	2.26	23.19	20.08	20.54	.67

TABLE E 3. LEAST SQUARES ANALYSIS OF VARIANCE AND MEANS OF EXCRETION OF PHOSPHORUS IN URINE (G/D).

Least Squares Analysis of Variance

	BALANCE 1				BALANCES 2, 3, & 4			
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
D	2	20	18	8391	2	156 53	4 63	0416
Cow(D)	9	5 03	.47	8413	9	152 25	13 71	0001
Balance					2	1 97	.80	4552
D*Balance					4	7 62	1 54	2042
Error	[illegible]	4 77			[illegible]	50 41		

Contrast	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
L vs M & H	1	19	35	.5699	1	62 33	3 69	0671
M vs H	1	0	0	.9811	1	91 86	1 11	778

Least Squares Means

Diet	Mean	SE	Mean	SE
L	52	35	.71	86
M	75	38	1.43	1 08
H	.79	32	4.21	.83

Least Squares Means

	Balance				
Diet	1	SE	2	3	4	SE
L	52	35	86	.61	63	39
M	75	61	1 38	1 36	1 54	51
H	.79	.32	3.37	4.31	4.36	.39

TABLE E.4. LEAST SQUARES ANALYSIS OF VARIANCE AND MEANS OF TOTAL EXCRETION OF PHOSPHORUS (G/D).

Least Squares Analysis of Variance

	BALANCE 1				BALANCES 2, 3, & 4			
0	2	323.92	.53	.6053	2	17324.06	121.49	.0001
Cow(D)	9	1049.87	5.25	.0627	9	641.68	1.53	.1331
Balance					2	828.43	9.46	.0003
0*Balance					4	314.36	1.80	.1448

L vs M & H	1	9.43	.08	.7826	1	8684.13	121.80	.0001

Least Squares Means

Diet	Mean	SE	Mean	SE
L	71.85	5.05	54.61	1.77
N	66.66	5.05	66.69	2.23
H	73.56	4.68	92.46	1.72

Least Squares Means

	Balance					
Diet	1	SE	2	3	4	SE
L	71.85	5.05	51.70	52.15	59.99	2.33
M	66.66	5.05	66.71	65.04	68.31	3.01
H	73.56	4.68	85.57	91.39	100.43	2.33

TABLE F-5. LEAST SQUARES ANALYSIS OF VARIANCE AND MEANS OF PRODUCTION OF MILK (KG/D)..

Least Squares Analysis of Variance

	BALANCE 1				BALANCES 2, 3, & 4			
Source	DF	MS	F	P	DF	MS	F	P
D	2	76.89	.94	.4260	2	111.21	.75	.4965
Cow(D)	9	368.22	22.18	.0001	9	664.79	38.64	.0001
Balance					2	39.58	10.35	.0002
D*Balance					4	52.77	6.90	.0002
Error	12	22.13			42	19.66		

Contrast	DF	MS	F	P	DF	MS	F	P
L vs M & H	1	73.99	1.81	.2116	1	105.34	1.47	.2567
Error		3.40						

Least Squares Means

Diet	Mean	SE	Mean	SE
L	19.93	2.26	19.00	1.80
M	23.23	2.26	21.77	2.26
H	24.06	2.26	21.79	1.75

Least Squares Means

	Balance					
Diet	1	SE	2	3	4	SE
L	19.93	2.26	18.45	19.36	19.17	.49
M	23.23	2.26	24.36	20.82	20.15	.63
H	24.06	2.26	23.07	21.01	21.30	.49

APPENDIX G
LEAST SQUARES ANALYSIS OF VARIANCE OF DIGESTIBILITY OF DRY MATTER AND EXCRETION OF PHOSPHORUS FOR DATA DESCRIBED IN CHAPTER 4

TABLE G-1. LEAST SQUARES ANALYSIS OF VARIANCE AND MEANS OF DIGESTIBILITY OF DRY MATTER (%).

Least Squares Analysis of Variance

	Full Model				Reduced Model			
Source	df	Type III SS	F	Pr > F	df	Type III SS	F	Pr > F
D	2	195.06	52	.6100	2	195.06	.49	.6100
Cow(D)	9	1690.11	14.58	.0001	9	1690.11	12.75	.0001
Period (P)	7	394.21	4.40	.0001	7	394.21	3.92	.0003
D*P	14	314.09	1.75	.0474	14	314.09	1.64	.0703
Method	2	2622.64	102.41	.0001	2	2588.01	94.41	.0001
D*Method	4	120.75	2.36	.0546				
P*Method	14	324.35	1.81	.0380				
Error	201	2571.33			239	3291.25		

Contrast	df	Type III SS	F	Pr > F	df	Type III SS	F	Pr > F
Coll vs other	1	2559.42	202.28	.0001	1	2547.82	185.99	.0001
Cr vs ADL	1	39.21	3.09	.1003	1	40.19	2.93	.0881

Least Squares Means

Method	Mean	SE	Mean	SE
Coll	62.64	.38	62.59	.39
Cr	55.65	.38	55.64	.39
ADL	56.51	.38	56.57	.39

Least Squares Means

	Period								
Method	1	2	3	4	5	6	7	8	SE
Coll	62.42	62.92	62.86	60.91	63.01	63.30	60.62	65.06	1.03
Cr	52.79	54.00	55.70	56.56	53.30	58.12	55.14	59.60	1.03
ADL	54.14	54.91	55.70	57.06	54.12	58.44	54.61	57.50	1.03

[illegible] SQUARES ANALYSIS OF VARIANCE AND MEANS OF ESTIMATION OF EXCRETION OF P IN FECES (G/D).

Least Squares Analysis of Variance

		Full Model			Reduced Model			
[illegible]	DF	[illegible]	F	PR	DF	[illegible]	F	PR
D	2	2689.29	24.63	.0002	2	2689.29	24.63	.0002
Cow(D)	9	4914.52	14.03	.0001	9	4914.52	13.57	.0001
Period (P)	7	8103.11	29.75	.0001	7	8103.11	28.77	.0001
D*P	14	13923.73	25.56	.0001	14	13923.73	24.72	.0001
Method	2	4865.83	62.52	.0001	2	4798.59	59.63	.0001
D*Method	4	290.95	1.87	.1168				
P*Method	14	734.29	1.35	.1811				
Error	[illegible]	[illegible]			238	5576.19		

[illegible]	DF	TYPE [illegible]	F	PR	DF	[illegible]	F	PR
Coll vs other	1	4824.28	123.98	.0001	1	4744.08	117.31	.0001
[illegible]	1	41.55	[illegible]	[illegible]	1	[illegible]	[illegible]	[illegible]

Least Squares Means

Method	Mean	SE	Mean	SE
Coll	49.66	.66	49.73	.67
Cr	59.09	.66	59.12	.67
ADL	58.13	.66	58.03	.67

Least Squares Means

	Period								
[illegible]	1	2	3	4	5	6	7	8	[illegible]
Coll	52.00	47.26	43.74	44.87	50.59	46.74	57.25	54.63	1.90
Cr	66.23	58.85	53.07	50.05	63.75	52.49	65.46	62.82	1.90
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

APPENDIX H
LEAST SQUARES ANALYSIS OF VARIANCE
FOR DATA DESCRIBED IN CHAPTER 5

TABLE H-1. LEAST SQUARES ANALYSIS OF VARIANCE AND MEANS OF AVERAGE DAILY INTAKE OF DRY MATTER (KG/D).

Least Squares Analysis of Variance

Source	DF	Type III SS	F	PR
Age	1	7.28	.31	.5839
Cow(Age)	22	518.49	6.51	.0001
Period	2	36.23	5.00	.0116
Treatment				
Phosphorus (P)	2	1.88	.26	.7724
Calcium (Ca)	1	1.94	.54	.4682
P*Ca	2	5.94	.82	.4476
Age*Period	2	1.76	.24	.7856
Error	39	141.22		

Contrast	DF	Type III SS	F	PR
L P vs other P	1	1.39	.38	.5391
M P vs H P	1	.43	.12	.7318
L Ca vs H Ca	1	1.94	.54	.4682

Least Squares Means

	P			
Ca	.33	.43	.54	Mean
.60	23.98	23.98	23.00	23.65
.97	24.14	23.67	24.20	24.00
Mean	24.05	23.82	23.60	

TABLE H 2. LEAST SQUARES ANALYSIS OF VARIANCE AND MEANS OF ACTUAL MILK YIELD (KG/D).

Least Squares Analysis of Variance

Source	DF	Type III SS	F	PR
Age	1	282.30	8.45	0082
Cow(Age)	22	735.43	22.90	.0001
Period	2	179.52	81.49	.0001
Treatment				
Phosphorus (P)	2	7.35	2.52	.0938
Calcium (Ca)	1	9.35	6.41	.0155
P*Ca	2	1.65	.57	.5727
Age*period	2	10.97	3.72	.0331
Error	39	56.93		

Contrast	DF	Type III SS	F	PR
L P vs other P	1	5.13	3.52	.0682
M P vs H P	1	1.96	1.34	.2536
L Ca vs H Ca	1	9.35	6.41	.0155

Least Squares Means

	P			
Ca	.33	.43	.54	Mean
60	23.36	22.62	22.08	22.69
97	22.14	22.02	21.61	21.93
Mean	22.76	22.32	21.85	

TABLE H-3. LEAST SQUARES ANALYSIS OF VARIANCE AND MEANS OF 3.5% FAT CORRECTED MILK (KG/D).

Least Squares Analysis of Variance

Source	DF	Type III SS	F	PR
Age	1	195.75	5.66	.0263
Cow(Age)	22	758.78	11.51	.0001
Period	2	166.15	27.73	.0001
Treatment				
Phosphorus (P)	2	6.05	1.01	.3737
Calcium (Ca)	1	15.15	5.06	.0303
P*Ca	2	1.97	.33	.7214
Age*Period	2	27.56	4.60	.0161
Error	39	116.85		

Contrast	DF	Type III SS	F	PR
L P vs other P	1	5.87	1.96	.1697
M P vs H P	1	.11	.04	.8462
L Ca vs H Ca	1	15.15	5.06	.0303

Least Squares Means

	P			
Ca	.33	.43	.54	Mean
.60	24.74	23.64	23.67	24.02
.97	23.28	23.05	22.79	23.04
Mean	24.01	23.35	23.23	

TABLE H-4. LEAST SQUARES ANALYSIS OF VARIANCE AND MEANS OF 4.0% FAT CORRECTED MILK (KG/D).

Least Squares Analysis of Variance

Source	DF	Type III SS	F	PR
Age	1	167.47	5.58	.0263
Cow(Age)	22	649.09	11.51	.0001
Period	2	142.14	27.73	.0001
Treatment				
Phosphorus (P)	2	5.17	1.01	.3736
Calcium (Ca)	1	12.96	5.06	.0303
P*Ca	2	1.69	.33	.7214
Age*Period	2	23.57	4.60	.0161
Error	39	99.95		

Contrast	DF	Type III SS	F	PR
L P vs other P	1	5.01	1.96	.1697
M P vs H P	1	.10	.04	.8461
L Ca vs H Ca	1	12.96	5.06	.0303

Least Squares Means

	P			
Ca	.33	.43	.54	Mean
.60	22.89	21.87	21.89	22.22
.97	21.54	21.32	21.08	21.31
Mean	22.21	21.59	21.49	

TABLE H-5 LEAST SQUARES ANALYSIS OF VARIANCE AND MEANS OF MILK FAT PERCENT.

Least Squares Analysis of Variance

Source	DF	Type III SS	F	PR
Age	1	.00011842	2.72	.1133
Cow(Age)	22	.00095786	5.53	.0001
Period	2	.00002354	1.50	.2366
Treatment				
Phosphorus (P)	2	.00000912	.58	.5649
Calcium (Ca)	1	.00000157	.20	.6571
P*Ca	2	.00000078	.05	.9514
Age*Period	2	.00002314	1.47	.2422
Error	39	.00030682		

Contrast	DF	Type III SS	F	PR
L P vs other P	1	.00000002	.00	.9563
M P vs H P	1	.00000912	1.16	.2883
L Ca vs H Ca	1	.00000157	.20	.6571

Least Squares Means

	P			
Ca	.33	.43	.54	Mean
.60	.0387	.0383	.0395	.0388
.97	.0387	.0380	.0389	.0385
Mean	.0387	.0382	.0392	

TABLE H-6 LEAST SQUARES ANALYSIS OF VARIANCE AND MEANS OF MILK PROTEIN PERCENT.

Least Squares Analysis of Variance

Source	DF	Type III SS	F	PR
Age	1	.00003955	3.24	.0854
Cow(Age)	22	.00026819	19.35	.0001
Period	2	.00003871	30.72	.0001
Treatment				
Phosphorus (P)	2	.00000038	.30	.7425
Calcium (Ca)	1	.00000572	9.08	.0045
P*Ca	2	.00000032	.25	.7791
Age*Period	2	.00000195	1.55	.2251
Error	39	.00002457		

Contrast	DF	Type III SS	F	PR
L P vs other P	1	.00000000	.00	.9841
M P vs H P	1	.00000046	.71	.4037
L Ca vs H Ca	1	.00000630	9.74	.0033

Least Squares Means

	P			
Ca	.33	.43	.54	Mean
.60	.0351	.0349	.0353	.0351
.97	.0345	.0345	.0353	.0345
Mean	.0348	.0347	.0349	

TABLE H-7 LEAST SQUARES ANALYSIS OF VARIANCE AND MEANS OF MILK LACTOSE PERCENT.

Least Squares Analysis of Variance

Source	DF	Type III SS	F	PR
Age	1	.00004852	8.91	.0071
Cow(Age)	22	.00012120	19.27	.0001
Period	2	.00000614	10.73	.0002
Treatment				
Phosphorus (P)	2	.00000018	.34	.7160
Calcium (Ca)	1	.00000027	.05	.3358
P*Ca	2	.00000008	.14	.8676
Age*Period	2	.00000024	.42	.6584
Error	39	.00001115		

Contrast	DF	Type III SS	F	PR
L P vs other P	1	.00000016	.55	.4619
M P vs H P	1	.00000003	.10	.7504
L Ca vs H Ca	1	.00000027	.95	.3358

Least Squares Means

	P			
Ca	.33	.43	.54	Mean
60	.0485	.0486	.0487	.0487
87	.0484	.0486	.0486	.0485
Mean	.0485	.0486	.0486	

TABLE H-8. LEAST SQUARES ANALYSIS OF VARIANCE AND MEANS OF CONCENTRATION OF P IN PLASMA (MG%).

Least Squares Analysis of Variance

Source	DF	Type III SS	F	PR
Age	1	10.90	4.66	.0420
Cow(Age)	22	51.45	2.14	.0186
Period	2	.97	.44	.6457
Treatment				
Phosphorus (P)	2	4.40	2.01	.1471
Calcium (Ca)	1	.15	.14	.7103
P*Ca	2	1.09	.50	.6115
Age*Period	2	1.21	.55	.5780
Error	39	42.65		

Contrast	DF	Type III SS	F	PR
L P vs other P	1	3.11	2.84	.0997
M P vs H P	1	1.45	1.33	.2567
L Ca vs H Ca	1	.15	.14	.7103

Least Squares Means

	P			
Ca	.33	.43	.54	Mean
60	7.23	7.66	7.27	7.39
97	6.74	7.77	7.35	7.29
Mean	6.99	7.72	7.31	

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BIOGRAPHICAL SKETCH

I, Deanne Morse, was introduced into the Selby Morse family on Bastille day in 1961, San Francisco, CA. My parents, brother, and I lived in Marin and Sacramento Counties until I was 11. At that time, my father accepted a principalship in Sonoma County, CA.

Through junior and senior high school, I was an active member of numerous clubs and organizations, especially the Future Farmers of America (FFA). Included in FFA judging activities were meats evaluation, parliamentary procedures, farm records, vine pruning, and public speaking. During my junior and senior year in high school, I assisted with a community college course in livestock and meat evaluation. In June, 1979, I received the FFA Community Service Award for a graduating senior.

Many high school interests were continued at the University of California, Davis, where I was a member of collegiate livestock, dairy, and meat judging teams as well as numerous committees appointed by the Agricultural Dean. In 1981, I interned with a Dairy Extension Agent in Fresno County, CA. This experience sparked a career interest in working for Cooperative Extension. In 1982, I was a proud recipient of FFA's American Farmer Degree. In May of 1983, I received the California Farm Bureau Federation Rural Leadership Award for being the one graduating college senior who showed the greatest promise of leadership in development of the farm and the farm home.

In August of 1983, I ventured to Gainesville, FL, for graduate training. My master's degree was in dairy science with emphasis on mastitis control and management of herd health records. My minor was food and resource economics. In May of 1986 I officially began study toward a Doctor of Philosophy degree with emphasis in dairy cattle nutrition. Minors for the Ph. D. are farming systems and agricultural and extension education. I have enjoyed assisting with laboratory courses and speaking with dairymen at dairy workshops. Currently, I am a candidate for a Doctor of Philosophy degree in animal science.

Once my degree requirements are fulfilled, I will be accepting a position as Extension Dairy Specialist for the state of North Dakota. Although I will leave many friends in Gainesville, I look forward to the new adventure in my life. After a six-year quest for knowledge in dairy cattle herd health and nutrition management, I will be delighted to continue my quest for knowledge with the assistance of the Extension Service.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

H. Herbert Head, Chairman
Professor of Dairy Science

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Harold H. Van Horn, Jr.
Professor of Dairy Science

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Barney Harris, Jr.
Professor of Dairy Science

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Professor of Animal Science

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Extension Education

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Edwin C. French, III
Associate Professor of Agronomy

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy

August, 1999

Jack L Fry
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